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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Direction of the Office of Management and Budget through January 31, 1983.

Acute Toxicity During Adjuvant Chemotherapy for Breast Cancer: The National Surgical Adjuvant Breast and Bowel Project (NSABP) Experience From 1717 Patients Receiving Single and Multiple Agents[1,2]

Andrew Glass, H. Samuel Wieand, Bernard Fisher, Carol Redmond, Harvey Lerner, Janet Wolter, Henry Shibata, David Plotkin, Roger Foster, Richard Margolese, Norman Wolmark, and Other NSABP Investigators[3,4,*]

SUMMARY

Since 1972, the National Surgical Adjuvant Breast and Bowel Project (NSABP) has carried out a series of clinical trials evaluating the worth of adjuvant chemotherapy in the management of patients with primary breast cancer. This report provides information concerning (*a*) protocol compliance relative to drug administration and (*b*) acute toxicity encountered by patients in three separate trials who were given one-, two- or three-drug chemotherapy within 1 month of operation. The findings are derived from 1548 women who received 20,765 courses of chemotherapy, the most extensively documented experience yet reported. They indicate that despite the large number of physicians and the heterogeneity of the institutions participating, large cooperative efforts can be accomplished with credibility. Only 13 (0.8%) of the women failed to complete all courses of therapy for reasons directly related to nonprotocol compliance by physicians. Only 4.3% failed to complete therapy for miscellaneous reasons other than toxicity, treatment failure, occurrence of a second primary, or death unrelated to tumor. While almost all patients experienced toxic reactions during the therapy, only 3%–4% of recipients of melphalan (L-PAM; P) and 4%–5% of recipients of L-PAM + 5-FU(F)(PF) failed to complete 2 years of therapy because of toxicity. Of those patients receiving PF + methotrexate (MTX; M) (PMF), 15% did not finish their treatment for that reason. While there was little difference in hematologic and nonhematologic toxicity between those patients receiving P or PF, and such toxicity was generally acceptable to both patients and physicians, the addition of MTX (PMF) resulted in greater toxicity (vomiting, stomatitis, and alopecia) which was less readily accepted. Tolerance of any of these regimens was unrelated to patient age, despite the belief that older women are less tolerant of chemotherapy. The earlier toxicity occurred, the greater was the number of subsequent courses associated with toxicity, and the lower was the total amount of drug received. The extent of the toxicity produced by the NSABP regimens and the end results obtained with them, must be compared with the end results and toxicity obtained by other regimens before making a choice of the adjuvant therapy to be used.

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[2]Supported by Public Health Service grant CA-12027 and contract N01-CB-23876 (Division of Cancer Biology and Diagnosis) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and American Cancer Society grant RC-13.

[3]*See Appendix* at end of paper.

[4]We acknowledge with gratitude the assistance of Elizabeth A. Saffer in the preparation of this manuscript.

**Reprint requests to:* NSABP Headquarters, 914 Scaife Hall, University of Pittsburgh, Pittsburgh, PA 15261.

Since 1972, a series of clinical trials has been carried out by the National Surgical Adjuvant Breast and Bowel Project (NSABP) to determine the validity of the rationale for the use of adjuvant chemotherapy in the management of patients with primary breast cancer. While the trials have been primarily oriented toward determining clinical benefit, they were conceived to test in an orderly fashion the hypothesis derived from experimental models that tumor cell populations between 10^6 and 10^7 cells may be amenable to single agent chemotherapy. It has been estimated that about 75% of women with stage II breast cancer harbor that number of residual cells following primary tumor removal. These patients should benefit by the use of a single agent, if that contention is correct. For that reason, the first of three consecutive NSABP clinical trials (B-05) evaluated the worth of a single agent. After determining its effectiveness, protocols (B-07 and B-08) which evaluated the worth of increasing numbers of drugs were implemented in stepwise fashion in an attempt to identify subsets of patients who might be as responsive to a single agent or to two agents as to more complex combinations of drugs. The investigations were also aimed at supplying information to indicate whether micrometastases respond to chemotherapy differently than does a greater tumor burden (advanced disease). In addition, they were carried out to determine whether the use of greater numbers of chemotherapeutic agents would produce better results that would counterbalance the putative greater toxicity of such therapy.

Relatively early results from these three NSABP trials have been reported (1–5). They demonstrated, depending upon the subset of patients being treated, the worth of postoperative systemic chemotherapy in variably decreasing the probability of treatment failure and augmenting survival. The findings also indicate that the increments of benefit obtained in certain patient subsets are remarkably similar to those noted employing other regimens, eg, CMF (6,7). The prior publications regarding the NSABP trials have not presented in detail the protocol compliance relative to drug administration or the incidence of acute toxicity encountered by patients on the various regimens. The purpose of this report is to provide toxicity information, since it could be a factor in determining the choice of adjuvant therapy to be administered to patients with breast cancer, particularly when similar benefits are achieved relative to decrease in treatment failure and improvement in survival by different regimens. The findings are derived from 1548 women who received 20,765 courses of chemotherapy.

PROCEDURE

Between September 1972 and April 1977, 1848 women were randomized into three consecutive NSABP trials of adjuvant chemotherapy (table 1). All women having a radical mastectomy (conventional or modified) for potentially curable breast cancer, having one or more axillary nodes proved histologically to contain tumor, and who were deemed free of detectable metastatic disease, were considered eligible for inclusion in the evaluations, provided they fulfilled specific criteria described in the protocols. Those criteria are identical for all protocols except that the age limit for eligibility was reduced from 75 years in B-05 and B-07 to 70 years in B-08. The specific conditions for patient eligibility and ineligibility have been previously reported. This report is comprised of data from 1717 women who were eligible for the three studies and for whom toxicity information was available. Excluding 169 patients in B-05 who received placebo, there were 1548 patients receiving 20,765 courses of chemotherapy.

In each of the studies, patients were randomized into one of two groups. Protocol B-05 compared melphalan (L-PAM; P) to placebo in double-blinded fashion. The second trial (B-07) tested P vs P + 5-FU (F) and the third (B-08) compared PF with PF + methotrexate (MTX; M). The dose of drugs and regimens employed are presented in table 1. Therapy was begun in all patients within 4 weeks of operation. The projected duration of therapy was 102 weeks (~2 years; 17 6-week cycles). If treatment had to be delayed so that the 17 courses could not be completed by 2 years, treatment was stopped at 2 years.

Almost all patients received their entire therapy while ambulatory. Only the rare patient needed to be hospitalized during the 5 days of treatment. The L-PAM was customarily taken in the evening. All doses of 5-FU and MTX were administered by rapid infusion using a "butterfly" needle and saline flush. Antiemetics were prescribed at the discretion of the attending physician. No other antineoplastic therapy was given prior to documentation of a treatment failure. Patients in the study had a physical examination every 6 weeks. Hematology testing was performed every 3 weeks (Days 1 and 21 of each cycle) and blood chemistry determinations were made every 12 weeks. The dose of chemotherapy was adjusted according to the level of total wbc and platelet counts on the starting day of each 6-week cycle of treatment. In B-05 and B-07, the blood cell counts obtained in the middle of the cycle (Day 21) were tabulated but not used in dose modification. In B-08, modifications were made in dosage despite

TABLE 1.—Schema of NSABP trials (B-05, B-07, and B-08) of adjuvant therapy

Protocol	Patient entry (date) Begun	Terminated	No. of patients*	Randomization	Drug regimen
B-05	9-27-72	2-05-75	170	Placebo	0.15 mg/kg/day orally × 5 days q6wks × 17
				vs	
			179	L-PAM (P)	0.15 mg/kg/day orally × 5 days q6wks × 17
B-07	2-05-75	5-15-76	346	P	6 mg/m²/day orally × 5 days q6wks × 17
				vs	
			325	P	4 mg/m²/day orally × 5 days q6wks × 17
				+	
				5-FU (F)	300 mg/m²/day iv × 5 days q6wks × 17
B-08	4-12-76	4-29-77	362	P	4 mg/m²/day orally × 5 days q6wks × 17
				+	
				F	300 mg/m²/day iv × 5 days q6wks × 17
				vs	
			336	P	4 mg/m²/day orally × 5 days q6wks × 17
				+	
				F	300 mg/m²/day iv × 5 days q6wks × 17
				+	
				MTX (M)	25 mg/m² iv — Day 1 + Day 5 q6wks × 17

*With toxicity information.

adequate blood cell counts on Day 1, if the counts on Day 21 were low. Greater than 90% of the patients had a blood cell count taken on Day 21.

The following dose modification scheme was used in all three studies.

Toxicity grade	*Wbcs/mm³*	*Platelets/mm³*	*% of drug given*
0	≥ 4000	≥ 100,000	100
1	2500–3999	75,000–99,000	50
2	≤ 2500	≤ 75,000	0

Patients who developed grade 2 toxicity were re-evaluated every 3 weeks until their blood cell counts rose to at least grade 1 toxicity levels, at which point treatment was resumed. In B-05, if patients experienced no toxic effects after three courses, the dose of L-PAM was increased (0.20 mg/kg/day). In B-07, if no toxicity occurred after three courses, the L-PAM was increased to 8 mg/m²/day in the arm receiving L-PAM only, and to 6 mg/m²/day in the PF arm. The 5-FU dose was not increased. There was no dose escalation in B-08.

All toxicity data were reported on standard NSABP forms completed following each treatment cycle. The amount of drug administered at a particular course and any other information concerning toxic events appeared on that form. All records were encoded at the NSABP Headquarters office so that they were in computer form. The present analysis is derived from these data.

RESULTS

Course Compliance by Physicians in Patients Starting Therapy

Physician compliance relative to therapy administration (not to amount of drug received) was evaluated in the 1548 patients who initially received adjuvant chemotherapy, whether or not they completed 17 courses (table 2). During the time in the protocol, these patients were eligible for the receipt of 21,062 courses of therapy. Of that number, 20,697 (98.3%) were correctly managed. In the 20,765 courses associated with grade 0 or 1 hematologic toxicity, therapy was given in 20,557 instances (99% compliance). A total of 297 courses were followed by grade 2 hematologic toxicity. The protocol stipulated that no therapy was to be given during those courses. Therapy was, however, administered in 157 instances so that protocol compliance was only 47%.

Incidence of and Characteristics, Course, and Drug Compliance of Patients Completing 2 Years of Chemotherapy

Of the 1548 patients who initially received adjuvant chemotherapy in the three protocols, 68% completed the 2 years of therapy (table 3). Little variation in the proportion completing therapy (~ 70%) was observed from one protocol to another with the possible exception of patients receiving PMF in B-

TABLE 2.—Course compliance during protocol

Course	Protocol					Totals
	B-05	B-07		B-08		
	P	P	PF	PF	PMF	
	Patients with grade 0 or 1 hematologic toxicity					
No.	2887	4403	4320	4924	4231	20,765
% compliance	99	98	99	99	99	99
	Patients with grade 2 hematologic toxicity					
No.	66	141	44	23	23	297
% compliance	53	44	43	52	59	47
	All patients starting therapy					
No.	2953	4544	4364	4947	4254	21,062
% compliance	98	98	98	99	99	98

TABLE 3.—Age and nodal status of patients completing 2 yrs of chemotherapy

Patients	Protocol					Total
	B-05	B-07		B-08		
	P	P	PF	PF	PMF	
No. starting therapy	179	346	325	362	336	1548
% completing therapy						
All	68	68	72	70	62	68
≤ 49 yrs	76	66	70	72	64	69
1–3 positive nodes	91	78	82	77	75	79
≥ 4 positive nodes	59	53	55	66	50	56
≥ 50 yrs	64	69	74	70	61	68
1–3 positive nodes	77	81	79	80	68	77
≥ 4 positive nodes	49	56	69	58	53	58

08. Only 62% of those women completed the therapy. The proportion completing treatment was unrelated to patient age alone, ie, ≤ 49 or ≥ 50 years; or to age with equivalent nodal status. In both age groups more patients having one to three positive axillary nodes completed therapy than did those with greater than or equal to four positive nodes, due to the greater incidence of treatment failure during therapy in the patients with greater than or equal to four positive nodes.

Almost all patients completing the 2 years of chemotherapy reported to their physicians for each of the 17 courses (table 4) whether or not, because of toxicity, therapy was to be given. Even patients given PMF were seen by their physicians at 94% of the courses. The total amount of drug received by those completing 2 years of therapy is presented in table 4. Also indicated is the percent of total drug (protocol amount) they would have received during 17 courses if there had been no dose reduction because of toxicity, ie, amount administered at the first course × 17. Patients in B-05 and in the PF arm of B-07 received > 90% of the protocol prescribed amount of P, while those in the P arm of B-07 received only 79% and those in PMF (B-08) were given 81% of the P.

Incidence of and Reasons for Patients Not Completing Protocol Prescribed Chemotherapy

Approximately one third of the patients in the various protocols failed to complete 2 years of therapy (table 5). The major reason for patients receiving P or PF being taken off protocol was the occurrence of a treatment failure. No more than 5% of patients failed to complete therapy because of toxicity. An equal proportion of those treated with PMF, on the other hand, were taken off protocol because of treatment failure (16%) and because of toxicity (15%). A small number of patients in any treatment group failed to complete therapy due to such miscellaneous reasons unrelated to toxicity as (*a*) patient refusal, (*b*) patient having moved, lost to followup, changed physicians who changed drugs,

TABLE 4.—Course and drug compliance in 1056 patients completing 2 yrs of chemotherapy

	Protocol				
	B-05	B-07		B-08	
Patients	P	P	PF	PF	PMF
No. completing therapy	122	235	235	255	209
% course compliance	98	98	98	95	94
Drug received					
P					
%*	92	79	95	84	81
mg/m²	421	404	322	286	278
F					
%	—	—	84	80	78
g/m²	—	—	21.4	20.5	20.1
M					
%	—	—	—	—	78
mg/m²	—	—	—	—	657

*Protocol amount.

TABLE 5.—% of patients not completing 2 yrs of therapy

	Protocol				
	B-05	B-07		B-08	
Reasons taken off protocol	P (57)*	P (111)	PF (90)	PF (107)	PMF (128)
% of patients					
Treatment failure	20.7	25.1	18.8	17.4	16.3
Second primary	2.8	0.3	1.5	0	0
Deaths without treatment failure	1.7	0.3	0.6	0.6	1.5
Toxicity	3.4	3.8	4.3	4.7	14.5
Miscellaneous (unrelated to toxicity or treatment failure)	3.4	2.6	2.5	6.9	5.6
Total	31.8	32.1	27.7	29.6	37.9

*Nos. in parentheses = No. of patients.

or (*c*) medical problems unrelated to the therapy or to breast cancer. The few patients in (*a*) (1%) who refused to continue therapy demonstrated no evidence to suggest that they did so because of toxicity. Their number was too few to permit independent analysis.

The frequency of patients failing to complete therapy because of toxicity was unrelated to age. A similar proportion of those ≤ 49 or ≥ 50 years of age, regardless of nodal status, were unable to complete therapy (table 6). There was no difference between the younger or older patients within a positive nodal category, ie, one to three or greater than or equal to four.

Relatively few patients failed to complete therapy because of hematologic toxicity (table 7). The major difficulty encountered was related to the gastrointestinal system. Two thirds of those patients receiving PMF who were taken off protocol because of toxicity received approximately one half of the protocol-stipulated number (17) of courses prior to that time (table 8). During that time the amount of drug received varied from 65% of P in patients in B-05 to 34% of M in patients receiving PMF in B-08.

Hematologic Toxicity

Incidence and severity.—Hematologic toxicity was evident following the first course of therapy in

a proportion of patients in all treatment groups (fig 1); a greater percent of patients receiving three-drug therapy experienced grade 1 or 2 toxicity than did those receiving one or two drugs. By the seventh course, there was little difference in the overall hematologic toxicity in any therapeutic group. Between 50% and 60% of patients experienced some degree of toxicity at any subsequent course. Only 10% of women receiving PMF experienced grade 2 toxicity at any course, in patients receiving PF or P, the incidence of grade 2 toxicity was even lower. Almost all women experienced grade 1 toxicity at some point, as is evidenced in the cumulative percent plots (fig 2). Almost 40% of those receiving PMF demonstrated grade 2 toxicity at least once by completion of the 2 years of treatment, whereas the percentage was less (20%–30%) for those given PF or P.

In the patients who completed 17 courses of therapy, there was a remarkable similarity between all treatment groups relative to the proportion of patients with the same frequency of toxicity (fig 3). Fifty percent of patients had greater than or equal to ten courses associated with toxicity, whether they received P, PF, or PMF. Two patients receiving PMF developed sudden severe leukopenia and rapidly died of unrecognized sepsis: one at 3 months and the other between the third and fourth months after the beginning of therapy.

Relation of time of onset to subsequent frequency.—The course when toxicity was first manifested was an indicator of the extent of subsequent toxicity. Those patients experiencing leukopenia or thrombocytopenia in the first three courses demonstrated a greater incidence of toxicity in subsequent courses than did those whose first toxic event occurred between the fourth and ninth course (fig 4). For example, ~ 50% of patients receiving P in B-07 who showed early toxic reactions had manifestations of toxicity in 75% of subsequent courses, whereas only ~ 10% of women who first developed toxic effects later in the treatment (fourth to ninth course) demonstrated the same frequency of courses associated with toxicity.

Relation to menopausal status.—When the cumulative percent of patients with hematologic toxicity by a particular course was charted for pre- and

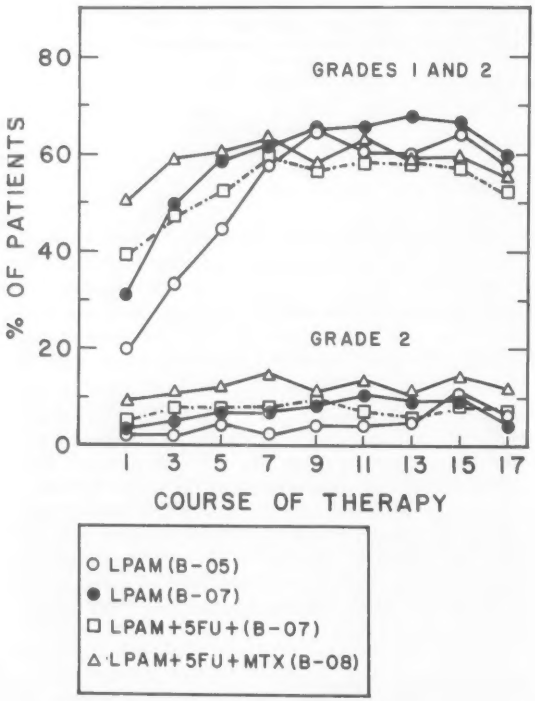

FIGURE 1.—% of patients with hematologic toxicity at a particular course of therapy.

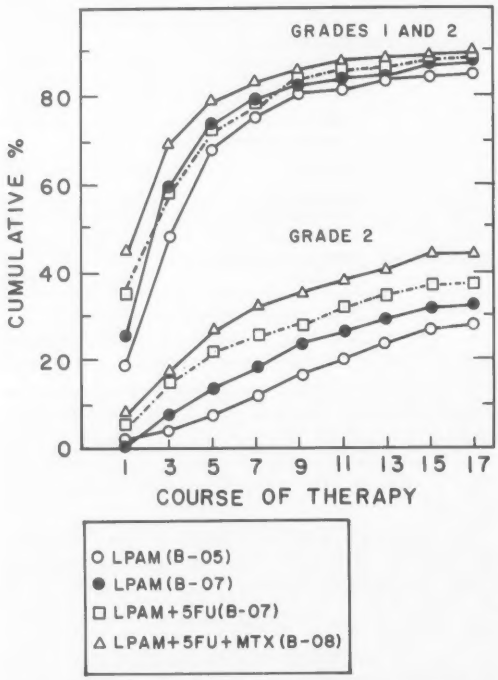

FIGURE 2.—Cumulative % of patients in study with hematologic toxicity by a particular course: all patients.

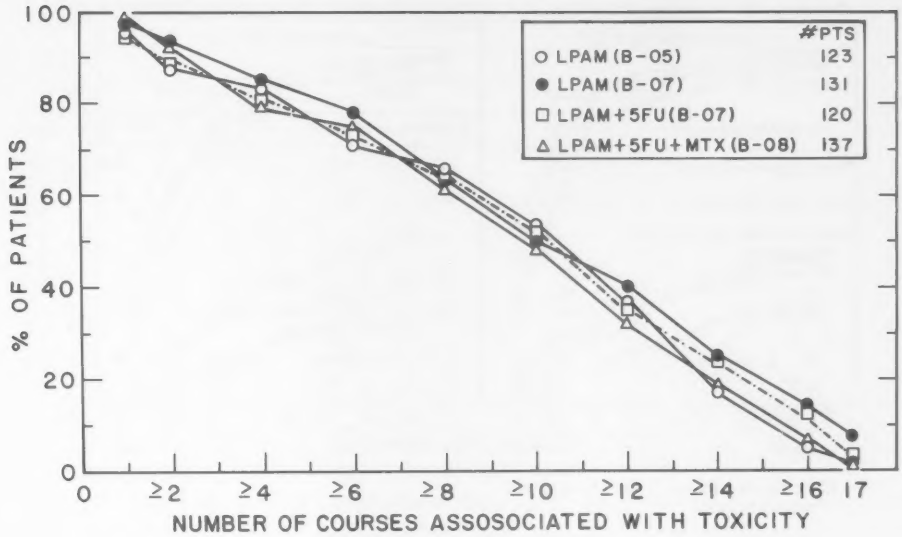

FIGURE 3.—Frequency of hematologic toxicity in patients completing 17 courses of therapy.

post-menopausal patients (fig 5), there was little difference in the degree of toxicity experienced by these two groups of women. While older women developed grade 1 leukopenia with slightly greater rapidity, this rarely progressed to grade 2 levels. Premenopausal women, particularly those given PMF, developed grade 2 toxicity to a greater extent than did the older patients. When the percent of patients with hematologic toxicity at a particular course of therapy was related to menopausal status, further evidence was obtained from each of the treatment groups to indicate that no greater toxicity occurred in the post-menopausal patients (fig 6).

Relation to drug received.—Toxicity affected the amount of drug received over time (table 9). As the

TABLE 6.—Mean age and % of patients within an age group taken off protocol due to toxicity

	Protocol				
	B-05	B-07		B-08	
Age (yrs)	P	P	PF	PF	PMF
Mean	48	52	41	50	53
% ≤ 49*	3.4	3.5	5.6	6.5	12.5
% ≥ 50*	3.3	4.0	3.3	3.6	15.8

*% of all patients in that age category.

TABLE 7.—Patients taken off protocol due to toxicity

	Protocol				
	B-05	B-07		B-08	
Patients	P	P	PF	PF	PMF
% with toxicity*	3.4	3.8	4.3	4.7	14.5
Hematologic	1.7	2.9	0.6	0	3.9
Gastrointestinal and other	1.7	0.9	3.7	4.7	10.7

*Of all patients in protocol.

TABLE 8.—No. of courses and amount of drug received by patients not completing therapy due to toxicity

Patients	Protocol				
	B-05	B-07		B-08	
	P (6)*	P (13)	PF (14)	P (17)	PMF (49)
Time in protocol (mos)	16.7	16.2	11.1	13.1	10.6
% of course compliance	73	62	46	52	43
Drug received before taken off protocol					
P					
%†	65	54	50	46	38
mg/m²	300	278	171	155	129
F					
%	—	—	38	42	36
g/m²	—	—	9.6	10.6	9.2
M					
%	—	—	—	—	34
mg/m²	—	—	—	—	287

*Nos. in parentheses = No. of patients.
†Protocol amount.

number of courses increased, the average amount of drug administered per course per patient decreased. The major decrease was observed in the earlier courses correlating with the increasing hematologic toxicity noted at that time (fig 1). Despite this observation, the reduction in the first year was relatively small in most instances. After 1 year of therapy (nine courses) the greatest decreases in amount of drug given were observed when P was employed in B-07 (21%) and when M was given in

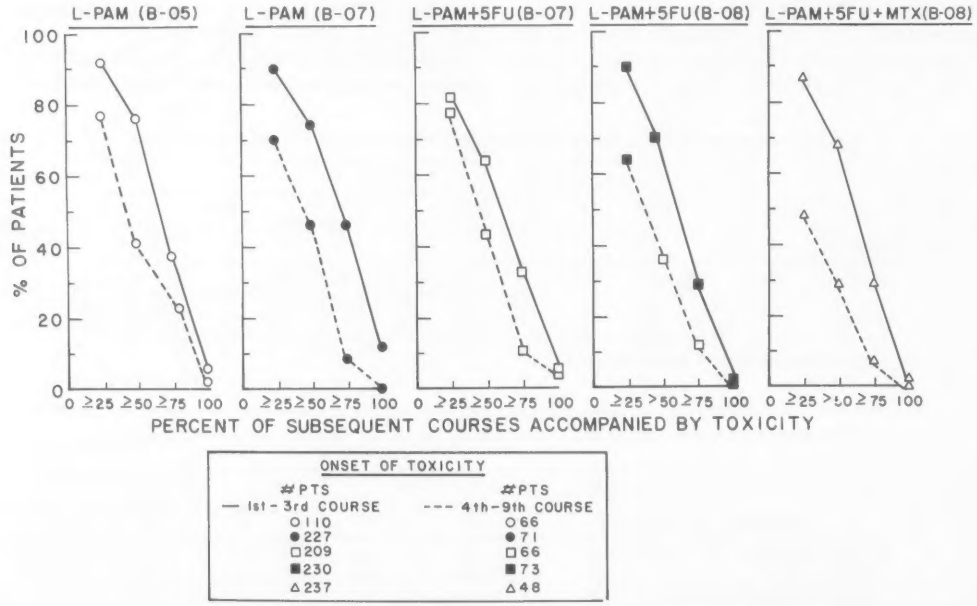

FIGURE 4.—Relation of time of onset of toxicity to subsequent frequency of toxicity.

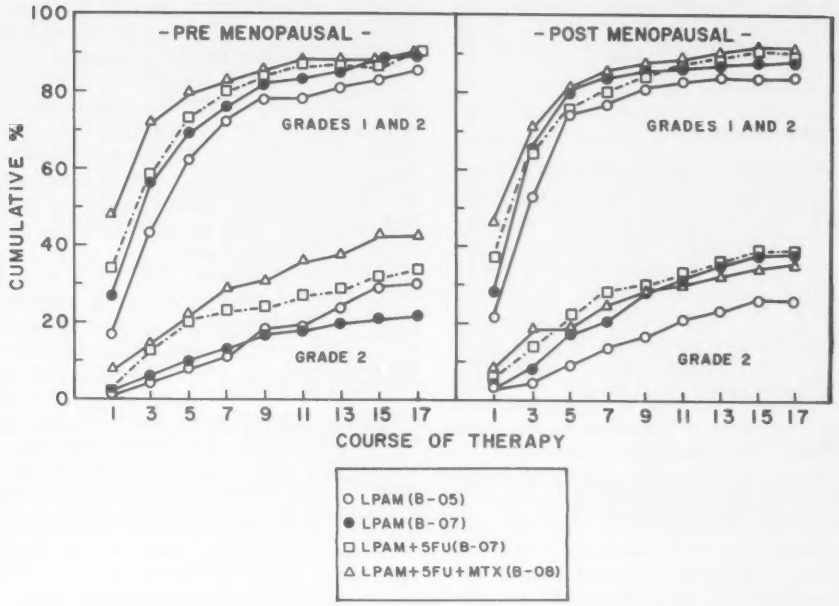

FIGURE 5.—Cumulative % of patients in study with hematologic toxicity by a particular course.

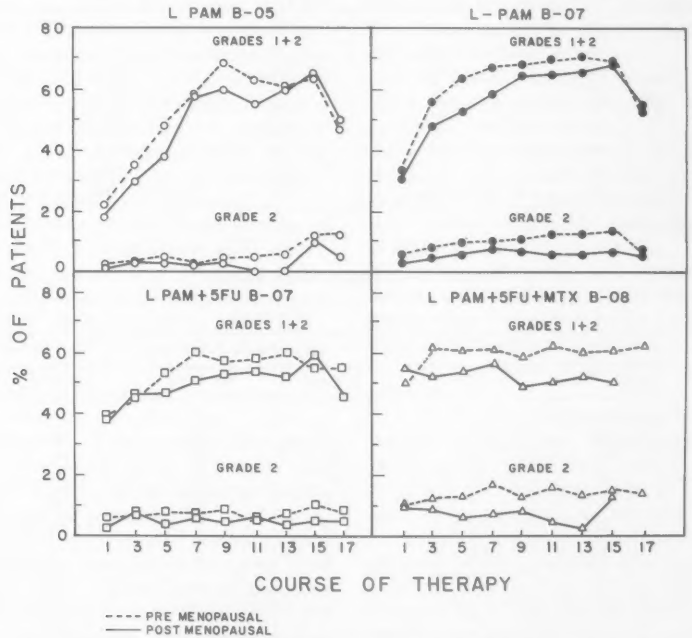

FIGURE 6.—% of patients with hematologic toxicity at a particular course of therapy: relation to menopausal status.

B-08 (24%). Little additional dose reduction occurred during the second year. Findings with P and F in B-08 (not included) were almost identical to those with the PF group in B-07.

Nonhematologic Toxicity

A significant number of patients receiving chemotherapy displayed some degree of nausea and vomiting (table 10). This became more severe with the administration of a greater number of drugs. Particularly severe vomiting developed with the three-drug combination. Stomatitis was also more common when MTX was administered. Alopecia was infrequent in the first two studies, but became more pronounced in B-08. Conjunctivitis and genitourinary problems were rarely encountered.

DISCUSSION

Because of breast cancer heterogeneity and the identification of increasing numbers of subsets of patients with different natural histories and responses to therapy, larger numbers of women than are available to any single investigator or institution are needed for trials of adjuvant therapy if answers are to be obtained within a reasonable time. Such a requirement has necessitated that many physicians from multiple institutions cooperate to attain the goals of a protocol. There has been serious reservation by many regarding how effectively and how uniformly such an effort could be carried out under such circumstances.

The present findings derived from > 1700 patients entered into protocols by several hundred physicians representing > 50 institutions in the US and Canada indicate that such cooperative efforts can be carried out with credibility. That only 4.3% of 1548 patients failed to complete therapy for miscellaneous reasons other than toxicity, treatment failure, occurrence of a second primary, or death unrelated to tumor, attests to that conclusion. Only 13 of the 1548 women did not complete all courses of therapy for reasons directly related to noncom-

TABLE 9.—Relation of toxicity to drug received*

Course No.	L-PAM, B-05	L-PAM, B-07	L-PAM in PF, B-07	5-FU in PF, B-07	MTX in PMF, B-08
1	47	52	36	2562	86
3	43	45	34	2417	76
5	45	46	37	2334	72
7	43	43	35	2204	67
9	42	41	34	2147	65
11	43	39	33	2090	64
13	40	38	33	2033	61
15	41	38	31	1955	62
17	39	37	31	1962	61

*Values = mg/course/patient.

TABLE 10.—Nonhematologic toxicity

	Protocol					
	B-05		B-07		B-08	
Toxicity	Placebo (170)*	P (179)	P (346)	PF (325)	PF (362)	PMF (336)
% of patients with—						
Nausea and vomiting	15	40	57	85	74	82
Mild	13	22	36	32	30	21
Moderate	2	12	16	37	30	36
Severe	0	6	5	16	14	25
Alopecia	2	1	3	6	14	22
Stomatitis	1	1	4	7	7	30
Conjunctivitis	1	0	1	<1	0	3
Genitourinary infection	2	1	2	1	0	2

*Nos. in parentheses = No. of patients.

pliance by physicians. They were dispersed about evenly in the five chemotherapy groups. This is particularly remarkable, in view of the relatively long duration of the therapy. Further indication that large numbers of physicians can comply with the requirements of a protocol is indicated by the finding that 99% of the courses that were to be given were administered. The reasons that some physicians administered drugs when grade 2 toxicity was present are not clear. Whether this was because the physicians tended to give a drug even in the face of the toxicity, rather than withhold treatment they believed to have possible benefit, or because of oversight, cannot be absolutely determined. While no untoward consequence resulted, such a finding mandates more constant, careful, continuing attention to protocols by participants.

Because of the diversity of the physicians and the heterogeneity of the institutions involved it is considered that these findings are, in all probability, more representative of the general experience with the use of adjuvant chemotherapy in the US and Canada than has yet been reported. Reports regarding the tolerance of patients and/or their physicians to toxic therapeutic regimens when treated by a single or a few physicians and institutions are less likely to be indicative of the overall state of affairs in that regard. Even within the NSABP studies, investigator bias in reporting nausea and vomiting could be discerned. The incidence of gastrointestinal toxicity varied greatly from institution to institution, suggesting substantial differences in the recognition of and/or emphasis given to patients' complaints. Some physicians seemed more willing than others to remove patients from treatment if nausea and vomiting became troublesome.

Almost all women experienced some degree of toxicity during therapy. In most it was mild, short-lived, and tolerable, particularly when they received P or PF. Only 3%–4% of recipients of the former and 4%–5% of recipients of the latter failed to complete the 2 years of therapy because of toxicity. On the other hand, 15% of those given PMF were unable to complete treatment for that reason. In those patients who did complete the 2 years of therapy, < 10% (2%–6%) of the courses of therapy in any treatment arm of the three protocols was missed because of toxicity. When patients did not complete therapy due to toxicity, those receiving PMF were taken off protocol sooner and completed fewer courses of therapy than did those given P or PF; consequently, they received less of the prescribed amount of drug for that protocol. It is important to point out, however, that patients who discontinued therapy because of toxicity still received a considerable amount of drug, since their "going off protocol" most frequently occurred in the second year of therapy. With increasing numbers of drugs this percentage was reduced somewhat so that those receiving PMF (B-08) received 43% of the planned courses of treatment. Patients given P in B-05 received 73% of the stipulated number of courses prior to cessation of treatment.

The death of two patients receiving PMF, which occurred in the early phase of the B-08 protocol, emphasizes the need for the most stringent surveillance of patients receiving adjuvant chemotherapy. With awareness of these incidents by all NSABP investigators and by institution of more rigorous vigilance, no further similar episodes occurred. There were several other instances of severe leukopenia or thrombocytopenia noted which were not accompanied by sepsis or bleeding.

The generally held belief that older women are unable to tolerate chemotherapy as well as younger women was not substantiated by these findings. No dose reduction at the start of treatment was made for older women and they required none. In all protocols, they tolerated the drugs as well as younger women. Their equivalent tolerance was not limited to the oral drug (L-PAM), but extended to the drugs given parenterally (5-FU and MTX), eliminating the possibility that they tolerated a drug because they were unable to absorb it and thus never experienced its toxic effects.

When toxicity occurred in early treatment courses there were a greater number of the subsequent courses associated with toxicity, and consequently, the lower was the amount of drug received. A comparison of the patients in B-05 given P with patients in B-07 given P exemplifies this finding. Those in B-07 started treatment with slightly higher doses of drug than did those in B-05. There resulted in B-07 earlier leukopenia and downward dose modifications in the subsequent courses. Moreover, patients who developed early leukopenia tended to display subsequent leukopenia. They thus tended to receive smaller doses of drug throughout their therapy.

Nonhematologic toxicity was the major reason for patients being taken off protocol due to toxicity, particularly in the PMF combination where vomiting was a serious problem. In general, the difference in nonhematologic toxicity between the one- or two-drug regimens was inconsequential. The addition of MTX produced effects not as readily tolerable. The vomiting, stomatitis, and incidence of alopecia with PMF became significant and were less readily accepted by patients and their physicians. Despite the relatively greater frequency of these

side effects after PMF, they were less common than has been reported when the CMF regimen has been employed (8).

All chemotherapeutic regimens employed today in the adjuvant setting are accompanied by variable degrees of toxicity. Since for the individual patient it is not possible to determine whether an adjuvant chemotherapy regimen is effective while it is being administered, toxicity has frequently been taken as the gauge for determining the dose and complexity of the drugs to be administered. It is unfortunate that this has given rise to the concept held by many that less toxic regimens are apt to be less effective in controlling tumor growth. To relate a priori the extent of toxicity with the benefit of an adjuvant therapy is inappropriate.

A principal aim of the NSABP over the years has been to ascertain whether, indeed, in certain subsets of breast cancer patients less toxic regimens could be equally as effective in preventing treatment failure as those producing greater toxicity. The present report has indicated the extent of toxicity produced by the NSABP regimens. These findings must be compared with the degree of toxicity and end results obtained by other regimens before making a choice of the adjuvant therapy to be used.

APPENDIX

List of NSABP Participants in Protocols B-05, B-07, and B-08

NSABP Institution	*Current Surgeon or Medical Oncologist*	*Current Pathologist*
Akron City Hospital	Marvin Sakol	L. B. Reyes
Albert Einstein (NY)	Herbert Volk	Boyce Bennett
Boston City Hospital	Arthur Glasgow	Leonard Berman
Boston University	Merrill Feldman	Philip Gordon
Daniel Freeman Hospital (CA)	Elliott Hinkes	Raouf Yuja
Dekalb General Hospital (GA)	S. Angier Wills	Frank Matthews
Denver General Hospital	George Moore	Joseph Preston
Downstate (SUNY)	Bernard Gardner	Anthony Nicastri
Ellis Fischel (MO)	William Johnston	Carlos Perez-Mesa
French Polyclinic (NY)	James McManus	Nijole Brazenas
Georgia Baptist Hospital	A. Hamblin Letton	Lester Forbes
Greater Bakersfield (CA)	James E. Donovan	Robert Wybel
Group Health Med. Ctr. (WA)	Robert Bourdeau	Charles Marshall
Hackensack Hospital (NJ)	Bernard Koven	Stuart Anderson
Harbor General Hospital (CA)	David State Yosef Pilch	Frank Hirose
Hennepin Co. General Hospital (MN)	Claude Hitchcock	John Coe
Hershey Medical Center (PA)	William DeMuth	Samuel Ward
Highland Hospital (NY)	Edwin Savlov	David Platt
Hotel-Dieu (Montreal)	Jacques Cantin	Yvan Boivin
Hotel-Dieu (Quebec City)	Louis Dionne	Real Lagace
Huron Road Hospital	M. D. Ram	Edward Siegler
Jewish General Hospital	Richard Margolese	Claude LaChance
Kaiser Permanente (Fontana, CA)	Vinod Patwardhan	Roy Hoke
Kaiser Permanente (Harbor City)	Eugene Pollack	John Rowe
Kaiser Permanente (Portland, OR)	Andrew Glass	Tsoi Tsuen Chan
Kaiser Permanente (San Diego)	Thomas Campbell	Marvin Nicola
Letterman General Hospital (CA)	Glenn Justice	Philip Stansifer
Louisiana State University	Isidore Cohn	Ronald Welsh
Lynn Hospital (MA)	Bernard Willett	Emei Shen
Medical College of Virginia	Walter Lawrence	Saul Kay
Medical College of Wisconsin	William Donegan	Lawrence Clowry
Memorial Ca. Res. Fdtn. (CA)	David Plotkin	Victor Rosen

List of NSABP Participants in Protocols B-05, B-07, and B-08 (cont'd)

NSABP Institution	*Current Surgeon or Medical Oncologist*	*Current Pathologist*
Mercy Hospital (IA)	Albert Clemens	Joseph Song
Metropolitan Hospital	John Weiksnar	Eugene Schwartz
Michael Reese Hospital	Richard Desser	Wendy Recant
Michigan State University (East Lansing, MI)	Leif Suhrland	Ralph Edminster
Montefiore Hospital (NY)	Richard Rosen	Norwin Becker
Montreal General Hospital	John MacFarlane	W. P. Duguid
Mount Sinai (Cleveland)	Sidney Sachs	E. Siegler
Newark Beth Israel (NJ)	Frederick Cohen	Alkiviadis Campbell
Oakland Naval Hospital (CA)	Captain R. M. Deaner	Philip Vogt
Pennsylvania Hospital	Harvey Lerner	Else Olen
Roger Williams General Hospital (RI)	Jack Sayran	Israel Diamond
Royal Victoria Hospital (Montreal)	Frank Cummings	Giles Tremblay
Rush-Presbyterian St. Luke's (IL)	Steven Economou	Edmund Pellettiere
Saginaw Cooperative Hospital (MI)	Nikolay Dimitrov	John Young
South Nassau Community Hospital (NY)	Nicholas LiCalzi	Ahmed Khapra
St. Luc Hospital (Montreal)	Roger Poisson	Claude Larose
St. Luke's Hospital (MO)	Paul Koontz	John Rippey
St. Vincent's Hospital (IN)	John Cavins	Lee Foster
St. Vincent's Hospital (NY)	Thomas Nealon	Ilona Toth
Tulane University	Edward Krementz Carl Sutherland	H. Nina Dhurander
University of Arkansas	Kent Westbrook	Glenn Baker
University of California (San Diego)	Yosef Pilch	Sidney Saltzstein
University of Illinios	Tapas Das Gupta	Jose Manaligod
University of Iowa	Richard Lawton Peter Jochimsen	Frederic Stamler
University of Louisville	Carl Knutson Condict Moore	William Christopherson
University of Maryland	E. George Elias	Cyril Toker
University of Mississippi	George Smith	Carl Evers
University of Pittsburgh	Bernard Fisher	Susan Rogers Edwin R. Fisher
University of Rochester	W. Bradford Patterson	David Platt
University of Texas, San Antonio	J. Bradley Aust Anatolio B. Cruz	George Bannayan
University of Vermont	Roger Foster	Roy Korson
University of Wisconsin	Douglas Tormey	Kennedy Gilchrist
USC-LA County Medical Center	Lewis Guiss	Peter Schwinn
US Naval Hospital (CA)	T. James Guzik	Adelbert Cramer
Valley Hospital (NJ)	Hugh Auchincloss	William O. Green
Washington University	Harvey Butcher Gordon Philpott	Walter Bauer
Wayne State University	Alexander Walt	Barbara Rosenberg
West Surburban Hospital (IL)	Everett Nicholas	Frederick Volini
White Memorial Med. Center (CA)	Samuel Fritz	George Kypridakis
Wilmington Medical Center	Robert Frelick	Patrick Ashley
Eastern Cooperative Oncology Group		
Allentown Hospital (PA)	David Prager	Paul Panas
Baptist Hospital (FL)	Edward Knight	Albert Drlicka
Berkshire Medical Center (MA)	Jesse Spector	Alex Parkinson

List of NSABP Participants in Protocols B-05, B-07, and B-08 (cont'd)

NSABP Institution	*Current Surgeon or Medical Oncologist*	*Current Pathologist*
Cleveland Metropolitan Hospital	Edward Mansour	John Reid
Chicago Medical School	Ediz Ezdinli	Osualdo L. Rubinstein
Georgetown University (DC)	Bruce Schnider	Dae Sok Koh
Jefferson Hospital	Arthur Weiss	Arthur Patchefsky
Jefferson Medical College	Harvey Brodovsky	Arthur Patchefsky
Lahey Clinic Foundation	Richard Oberfield	Melvin Legg
Ontario Cancer Foundation	David Klaassen	E. Lieta
Paris	Lucien Israel	Coston Brard
Tufts-Pondville Hospital (MA)	Leo Stolbach	Carol Amich
University of Alberta	Pierre Band	Gordon Bain
University of Manitoba	Martin Levitt David Bowman	M. P. Hamonic Donald Penner
University of Pennsylvania	John Glick	Horatio Enterline
Central Oncology Group		
Duke University (NC)	William Shingleton	Bernard Fetter
Ohio State University	John Minton	William Holaday

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Combination of High-Dose Cyclophosphamide, BCNU, and VP-16-213 Followed by Autologous Marrow Rescue in the Treatment of Relapsed Leukemia[1,2]

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SUMMARY

The combination of high-dose cyclophosphamide, BCNU, and VP-16-213 followed by autologous marrow rescue was evaluated in the treatment of relapsed leukemia refractory to normal-dose chemotherapy. Cyclophosphamide was given at a total dose of 4.5 g/m², BCNU at a dose of 300 mg/m², and VP-16-213 at a dose of 600 mg/m². Seven high-dose treatments followed by marrow rescue were administered to six patients. Two patients achieved complete remissions, three had partial remissions, and one achieved a minimal response. The toxicity of this regimen was moderate.

[Cancer Treat Rep 65:377–381, 1981]

Autologous bone marrow transplantation was proposed for relapsed leukemia as early as 1959 when McGovern et al (1) reported successful reinduction of a patient with lymphocytic leukemia treated with total-body irradiation (TBI) and infusion of autologous stored bone marrow. Several authors have since explored this approach for the treatment of relapsed acute leukemia and chronic myelogenous leukemia in blast cell crisis (2–5). We have recently reported a study treating relapsed acute leukemia with piperazinedione and TBI followed by infusion of stored remission bone marrow (6). Complete remissions (CR) were achieved in 46% of 24 patients. Their remission duration lasted from 2 to 14 months. Unresolved problems with this approach include the potential of contamination of remission bone marrow with leukemic cells and a possible decrease in transplantation potential of bone marrow stem cells exposed to long-time chemotherapy. Utilization of ablative chemotherapy, including TBI, limits the procedure of a one-time rescue operation. The introduction of high-dose chemotherapy and bone marrow rescue in relapsed leukemia might give additional therapeutic advantages. It allows for repeated administration of high-dose regimens which could be followed by conventional-dose chemotherapy in an attempt to kill leukemic cells transfused with the autologous marrow.

We have investigated the combination of high-dose cyclophosphamide, BCNU, and epipodophyllotoxin VP-16-213 (CBV) in doses severalfold higher than those applied with conventional treatment. Bone marrow was obtained in previous remissions. Six patients with acute lymphoblastic, acute undifferentiated, acute myeloblastic, and lymphoma cell leukemias were given transplants following this high-dose chemotherapy after they were proven resistant to available conventional treatment. This pilot study reports the response of this drug combination and the observed toxicity in a heavily pretreated patient population.

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MATERIALS AND METHODS

Informed Consent

All patients or their guardians signed informed consents, agreeing to the procedure of bone marrow aspiration and storage as well as to the administration of chemotherapy.

Patient Population

Six male patients received a total of seven courses of high-dose combination chemotherapy followed by autologous bone marrow rescue. The median age was 25 years (range, 21–40). The group consisted of patients with the following diagnoses: acute lymphoblastic leukemia (two patients), lymphoma cell leukemia (two), acute myeloblastic leukemia (one), and acute undifferentiated leukemia (one). Patients entered in this protocol were considered too high risks for an alternative autologous transplantation protocol utilizing piperazinedione and TBI because of poor performance status, previous treatment with maximal doses of anthracyclines, or questionable quality of stored remission bone marrow due to a suspected higher degree of contamination with leukemic cells (time from storage to relapse < 3 months). In one patient, allogeneic transplantation from an HLA-identical sibling following piperazinedione and TBI was planned if the patient would have reached a CR following CBV treatment. Patient 1 was treated twice for extramedullary disease in his fourth relapse using high-dose chemotherapy and marrow rescue. Five patients were treated in first and second relapse after failing one or two reinduction attempts. The performance status varied between 1 and 4. Patient 2 received high-dose chemotherapy and autologous bone marrow rescue when in a coma due to multifocal brain involvement.

Bone Marrow Aspiration and Storage

Bone marrow aspirations were carried out while the patients were in first and second remissions. Cells were collected under general anesthesia by multiple bone marrow aspirations from the posterior iliac crests. Between 1500 and 2500 ml of bone marrow aspirate was harvested and suspended in Hanks' balanced salt solution (HBSS) with preservative-free heparin. After the collection, a buffy coat preparation was done. The cells were stored according to the modified technique of Schaefer and Dicke (7).

At the time of transplantation, the ampules were thawed rapidly in a 50°C water bath. Immediately after thawing, HBSS equivalent to 1/50, 1/25, 1/12, etc of the ampule volume was added dropwise until the original volume was diluted tenfold. After stepwise dilution, the cells were centrifuged, resuspended in HBSS, and filtered through G-2 glass filters. Viability of the cells was tested by the assay for colony-forming units in culture (8). All patients were premedicated iv with 50 mg of diphenhydramine hydrochloride (Benadryl) and 250 mg of methylprednisolone (Solu-Medrol).

Chemotherapy

High-dose CBV was administered over 4 days. Bone marrow was reinfused 6–7 days after BCNU treatment. Cyclophosphamide was given daily at a dose of 1.5 g/m² in 250 ml of dextrose over 3 days. VP-16-213 was given twice daily at a dose of 75 mg/m²/injection in 250 ml of normal saline over 2 hours for 4 days. A total dose of 600 mg/m² of VP-16-213 was delivered. BCNU was given at a single dose of 300 mg/m² in 250 ml of dextrose over 60 minutes. Patient 1 received a second treatment with high-dose chemotherapy and autologous bone marrow rescue after full recovery from the first autologous transplantation.

Supportative Care

The patients were treated with prophylactic antibiotic combinations consisting of oral Bactrim and iv tobramycin as soon as the granulocyte cell count fell to < 500/mm³. When fever occurred, antibiotics were changed accordingly. If fungus infections were suspected, miconazole or amphotericin B was added. If no response was seen within 2–3 days, leukocyte transfusions from related family members were given until defervescence or recovery of granulocytes occurred. Platelet transfusions were given prophylactically when platelet counts were < 20 × 10^9/liter. All blood products were irradiated with 2500 rad prior to transfusion.

Characteristics of Infused Bone Marrow

The time from storage to relapse ranged from 2 to 18 months. Bone marrows were stored 4–23 months. Cell numbers ranging from 0.5 to 1.3 × 10^8/kg and colony-forming units in the spleen ranging from 3840 to 14,820/kg were infused.

RESULTS

The individual responses and response duration are shown in table 1. The response durations are

TABLE 1.—Response to CBV

Patient No.	Response	Response duration (mos)	Subsequent treatment	Survival (mos)
1	CR	6	Mechlorethamine, L-asparaginase, methotrexate, piperazinedione, TBI	7 (sepsis, pneumonia)
2	CR	7	—	8
3	PR	2	—	4
4	PR	3	AMSA	4
5	< PR	1	AMSA	2 (disseminated fungus)
6	PR	4	AMSA, OAP*, L-alanosine	12

*OAP = vincristine, cytarabine, and prednisone.

counted from the time high-dose chemotherapy was administered until relapse. Survival was measured from initiation of high-dose chemotherapy until death. Two patients achieved CR. Patient 1 had a complete disappearance of a leukemic renal mass documented by iv pyelogram, sonography, computerized axial tomographic scan, and renal biopsy. He received a second high-dose treatment 2 months later followed by stored remission bone marrow for consolidation. Patient 2, who had partially responded to Rubidazone, achieved a CR of 7 months' duration with CBV. In this case, the treatment modality was chosen because this patient had life-threatening multifocal chloromatous brain involvement. Cranial irradiation was administered simultaneously with chemotherapy. Three patients achieved partial remissions (PR) lasting from 2 to 4 months. Patient 5 had a minimal response leading to transient aplasia and partial normalization of blood cell counts for 1 month.

Hemopoietic suppression occurred during the first week of high-dose combination chemotherapy. Neutrophil recovery, > 500/μl, was observed at a median of 33 days following chemotherapy or 26 days following marrow infusion (range, 26–55 days after chemotherapy and 19–49 days after marrow infusion). Platelet recovery to a level of 20,000/mm^3 was reached at a median of 30 days after initiation of chemotherapy (range, 25–51 days). The total time of severe myelosuppression where antibiotics, blood products, and hospitalization were needed lasted 3–4 weeks.

Table 2 shows the toxic effects seen with this regimen. All patients had some degree of nausea and vomiting. Stomatitis was seen in four of seven courses, severe enough to require iv alimentation in one patient. Transient hepatic function abnormalities of moderate degree were observed in three patients. Hematuria occurred in two patients; patient 1, who had hematuria during the first course of high-dose treatment, did not develop hematuria during the second course of the treatment. Mild transient renal function abnormalities were seen in one patient and probably were related to a suspected generalized fungus infection. One patient developed spasms of 1 hour's duration in both legs on the third day of chemotherapy. Neurologic examination, EEG, and electrolytes including calcium, magnesium, and phosphate were within normal limits. All patients had fever during the period of neutropenia. One patient developed *Candida* esophagitis and *Pseudomonas aeruginosa* septicemia responding to systemic antibiotic and amphotericin treatment. Patient 3 developed a herpes simplex infection around the nostril 2 weeks after high-dose chemotherapy.

TABLE 2.—Toxic effects with CBV treatment

Toxic effect	Incidence in 7 courses
Gastrointestinal	
Stomatitis	4
Nausea and vomiting	7
Hepatic function abnormalities	3
Genitourinary	
Hematuria	2
Renal function abnormalities	1
Neurologic	1
Infections	
Fever of unknown origin	6
Bacterial (*Pseudomonas aeruginosa*)	1
Fungal (*Candida* esophagitis)	1
Viral (herpes simplex)	1

DISCUSSION

This paper describes our experience with high-dose CBV followed by autologous marrow rescue in the treatment of relapsed leukemia resistant to

conventional chemotherapy. All six patients showed bone marrow hypoplasia and evidence of hemopoietic recovery. The effect of the infused bone marrow cells on hemopoietic recovery cannot be evaluated clearly after nonablative treatment. Hemopoietic recovery can be due to infused cells or stem cells surviving chemotherapy or a combination of both. In a previously reported study using TBI, we have shown that cryopreserved remission bone marrow cells have the ability to rescue hemopoiesis after a stem cell ablative radiation dose (6). Other authors have shown that autologous marrow infusions can enhance hemopoietic recovery after nonablative high-dose chemotherapy (9–11). The aim of this pilot study was to explore the feasibility and activity of this high-dose drug combination in relapsed leukemia where other modalities were less promising. Two patients obtained CR lasting 6 and 7 months. PR of 2, 3, and 4 months' duration occurred in three patients.

The CBV combination, which had been originally designed for the treatment of oat cell carcinoma, appears effective in adult leukemias (10). High-dose cyclophosphamide, alone or in combination with other chemotherapy, is a standard treatment for relapsed leukemia and has led to long-term remissions in spite of the low activity seen by cyclophosphamide given in conventional doses (2,3,9,11–13). Normal-dose BCNU is of limited activity in acute leukemia; however, it has some activity in CNS leukemia, and increased doses of BCNU with marrow rescue are possible without additional toxicity (14). The combination of cyclophosphamide and BCNU has been explored in allogeneic transplantation by Graw et al (13). VP-16-213 has activity in acute leukemia, particularly of the acute nonlymphocytic type (15,16).

The toxicity of CBV in patients with solid tumors (10) was similar to that seen in the present study. More morbidity was seen in patients who had undergone extensive prolonged chemotherapy prior to entering the autologous bone marrow transplantation program. Patients who had less preceding therapy had faster hemopoietic recovery and less organ toxicity. Hemopoietic recovery in these six patients with leukemia was slower (neutrophils > 0.5, > 1.0, and $> 1.5 \times 10^9$/liter at 32, 47, and 49 days after chemotherapy, respectively; platelets > 20, > 50, and $> 100 \times 10^9$/liter at 30, 33, and 43 days after chemotherapy, respectively) than that observed in CBV-treated patients with solid tumors in whom median neutrophil recoveries of 0.5, 1.0, and 1.5 $\times 10^9$/liter were seen at 24, 26, and 27 days, respectively, and platelet recoveries of > 20, > 50, and $> 100 \times 10^9$/liter were seen at 21, 30 and 32 days, respectively, after the start of chemotherapy. The slower recovery in our patient population reflects the more extensive chemotherapy exposure of bone marrow cells in patients with leukemia.

It appears that high-dose CBV has limited nonhemopoietic toxicity and can even be administered twice consecutively followed by autologous marrow rescue. The responses of these heavily pretreated patients were promising. It is conceivable that the response rate will be higher in less intensively treated patients. The moderate observed morbidity allows one to consider this high-dose combination regimen for an intensification program in remission.

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Administration of BCG Cell Wall Skeleton Into Malignant Effusions: Toxic and Therapeutic Effects[1,2]

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SUMMARY

Thirty-nine patients with 40 refractory malignant effusions (26 pleural and 14 peritoneal) were treated locally with a nonviable mycobacterial vaccine. The vaccine was administered into the effusion and consisted of BCG cell wall skeleton and trehalose dimycolate attached to oil microdroplets. A dose range of 150–3000 μg was tested. The overall response rate was 44.0% (complete response [CR] plus partial response [PR]) and was not clearly dose-related. The response rates for each site were 13.6% (CR) and 31.8% (PR) for pleural effusions and 33.3% (CR) and 8.3% (PR) for peritoneal effusions. Toxic effects consisted of fever (40%), serosal pain (37.5%), and increased effusion (27.5%) and were not clearly dose-related. Gastrointestinal toxic effects were seen in 50% of patients treated for peritoneal effusions. Response correlated with prior exposure to BCG vaccine or tuberculosis, and with a febrile response to vaccine administration. This vaccine has a therapeutic effect on both pleural and peritoneal effusions.

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There has been recent interest in exploiting the local and regional effects of microbial vaccines in the treatment of malignancies at noncutaneous sites. McKneally et al (1) have reported a decreased relapse rate and prolongation of survival of patients with stage I non-oat cell carcinoma of the lung treated postoperatively with intrapleural BCG. Holmes et al (2) have recently reported a successful feasibility study of the transthoracic injection of BCG into primary lung carcinomas prior to their removal. Nonviable extracts of BCG have been investigated in the hope that they might provide standardized preparations and a greater therapeutic index. Yamamura et al (3) have treated patients with lung carcinoma with a nonviable BCG extract called BCG cell wall skeleton (CWS) attached to oil microdroplets. Patients with stage I, II, and III lung carcinoma of all major histologic types were treated with CWS as a supplement to conventional therapy. Their survival was superior to that of historic controls receiving conventional therapy alone. In addition, 55 patients with malignant pleural effusions were treated intrapleurally with CWS and this group also experienced a superior survival to that of historic controls treated with conventional therapy alone. We have reported the efficacy and toxicity of a similar product, BCG CWS and trehalose dimycolate (TDM) attached to oil droplets, in treating cutaneous and subcutaneous metastases in patients with breast cancer and melanoma (4).

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The combination of CWS-TDM was chosen because of its apparently greater tumor regressive activity, compared with that of CWS alone, in the line-10 guinea pig hepatoma model (5). The present study was designed to obtain preliminary information about the efficacy and toxic effects of the CWS-TDM-oil preparation in the treatment of malignant pleural and peritoneal effusions in patients with advanced refractory malignancies.

PATIENTS AND METHODS

All patients had histologic proof of malignancy and cytologic proof of malignant cells in the effusion. To be eligible for this study, the effusion must have recurred after at least one drainage procedure and must have proven refractory to first-line chemotherapy appropriate for the disease. Patients with visceral disease at other sites were accepted. Patients who had received prior immunologic therapy with BCG vaccine were also accepted. Patients concurrently receiving phase II chemotherapy were acceptable provided the effusion showed no response to chemotherapy, defined as shrinkage after at least one course of chemotherapy and measured at least 21 days following drug administration. Phase II chemotherapy was defined as chemotherapy or hormonal therapy with any degree of known efficacy in the disease being treated. Patients concurrently receiving phase I chemotherapy were also accepted. Phase I therapy was defined as chemotherapy with agents undergoing toxicity studies and having no known efficacy in breast cancer. A demonstrated failure of the effusion to respond to one course of phase I chemotherapy was not necessary for eligibility. For patients being treated with chemotherapy, vaccine treatments were intercalated between chemotherapy courses, allowing at least 1 week from the last dose of chemotherapy until administration of the vaccine and at least 2 weeks from the vaccine administration until the next dose of chemotherapy. Patients receiving chemotherapy were called responders to the immunotherapy only when the effusion itself responded and other measurable disease remained stable or increased. Patients in whom the effusion responded and other measurable disease simultaneously responded with a complete response (CR), partial response (PR), or less than a PR were considered to be unevaluable for treatment results.

In patients with pleural effusions, an attempt was made to drain as much of the effusion as possible by needle or catheter prior to vaccine administration. The vaccine was then injected back through the drainage device in 20–50 ml of normal saline. The patients then changed position every 5 minutes for 30 minutes to distribute the vaccine. Most of the patients were treated as outpatients. A CR was defined as no recurrence of effusion following drainage and treatment for at least 2 months. A PR was defined as a $< 50\%$ recurrence in the fluid as measured by height of the effusion from the diaphragm on chest roentgenogram. For patients with peritoneal effusion, no attempt was made to completely drain the effusion. Usually 1–3 liters were removed; the vaccine was then injected in 15–20 ml of saline, and the patient was directed to change positions as done for pleural effusions. A PR was defined as the loss of 5 cm in abdominal circumference for at least 2 months without complete resolution of the effusion. A CR was defined as the loss of at least 5 cm in abdominal circumference and no ability to detect effusion by physical examination. Response duration criteria were the same for peritoneal effusions as for pleural effusions. Baseline growth measurements used for evaluation of response were measured following the initial removal of fluid and/or following the removal of any "inflammatory response" fluid in the first 72 hours. Following instillation of the vaccine, patients were serially questioned and examined for pain and fever. Fever was considered present when temperature was $> 100°F$. Pain was considered present when the patient complained of this as a significant problem and required analgesics. Pleural effusions were examined by upright and decubitus roentgenograms. Abdominal effusions were followed by physical examination, including abdominal circumference measurements.

Some patients experienced a rapid accumulation of fluid within 72 hours following administration of the vaccine (*see* the Results section). This was interpreted as an inflammatory response to the vaccine, was drained, and did not influence subsequent interpretation of the response. Reaccumulation of fluid beyond this 72-hour point defined failure. All patients who received at least one dose of vaccine and who had at least 2 weeks' followup were considered evaluable. Statistical analysis was performed using chi-square tests with Yates' correction.

The BCG CWS-TDM-oil was prepared as previously described (5). The components were combined at a concentration of 750 μg of CWS and 750 μg of TDM (total, 1500 μg)/ml of vaccine. The vaccine was stored at 4°C and was tested for sterility; the unused portions were discarded after 2 weeks.

RESULTS

A total of 39 patients were treated. One of these patients had both pleural and peritoneal effusions and was therefore counted twice in this analysis,

TABLE 1.—Patient status according to site of effusion*

Characteristic	Total	Site of effusion	
		Pleural	Peritoneal
No. of patients	40	26	14
Sex			
Males	15	6	9
Females	25	20	5
Evaluable			
Toxic effects	40	26	14
Response	34	22	12
Other sites of metastases			
Visceral	26	13	13
Nonvisceral	5	4	1
None	9	9	0
Concurrent chemotherapy			
Phase II	30	20	10
Phase I	4	2	2
None	6	4	2
Prior BCG or tuberculosis			
Yes	19	16	3
No	21	10	11

*Values = No. of patients.

resulting in a total of 40 patients (table 1). Most of the patients were women with pleural effusions. Six patients experienced responses outside the locally treated site. For the purposes of this analysis, these responses were assumed to be due to concurrent chemotherapy. These patients were considered to be evaluable only for toxic effects. Most of the patients had tumor at nonpleural sites and had received prior BCG or had a clear history of active tuberculosis. Most of the patients with pleural effusions (69%) had breast carcinoma (table 2). Patients with peritoneal effusions had predominantly adenocarcinomas, primarily in the abdomen. Seven patients did not survive 2 months but were evaluable by the criteria stated in the Patients and Methods section.

Table 3 shows the number of patients and responses at each dose level. Of 34 evaluable patients, there were seven with a CR (20.6%) and eight with a PR (23.5%), for an overall response rate of 44.1%. There was no clear correlation between dose and response. Of the six patients receiving no chemotherapy, there were two PRs and one CR (total response rate, 50%). Of the four patients receiving phase I chemotherapy, there was one PR. The relation between dose and toxicity is shown in table 4. Nearly all toxicity was of < 72 hours' duration. The highest temperature (103°F) was recorded in one responding patient. Five patients (four responders) experienced prolonged fevers that lasted from 2 to 16 weeks; no cause other than toxicity could be discerned and the fevers gradually resolved without specific therapy. Twelve of 15 patients who responded experienced fever, whereas only four of 19 nonresponders experienced fever ($P < 0.01$). Eleven of the 15 responders had received prior BCG or had had tuberculosis, whereas this was true for only five of the 19 nonresponders ($P < 0.02$). Again, there was no clear correlation with dose. There was no significant correlation between pain or increased infusion and response.

TABLE 2.—Diagnosis according to site of effusion

Primary diagnosis	Site of effusion (No. of patients)	
	Pleural	Peritoneal
Breast	18	3
Adenocarcinoma (abdominal)	2	8
Lung (non-oat cell)	2	0
Mesothelioma	1	1
Melanoma	1	1
Other	2	1
Total	26	14

The relationship of response and toxicity to site of treatment is shown in tables 5 and 6. Gastrointestinal toxicity, which included nausea, vomiting, and diarrhea, is shown here. The overall response rates for pleural and peritoneal sites were approximately equal, although there was an apparently higher CR rate for pleural effusions. The frequency of toxicity was lower for peritoneal compared with pleural effusions, with the exception of added gastrointestinal toxicity. The only other toxic effect not

TABLE 3.—Response as a function of dose

Dose (μg)	Total	No. of patients		
		CR	PR	Failed or stable disease
150	3	0	2	1
300	6	3	1	2
600	5	0	1	4
750	4	0	0	4
1000	3	0	1	2
1500	8	1	3	4
3000	5	3	0	2
Total (%)	34	7 (20.6)	8 (23.5)	19 (55.9)

TABLE 4.—Toxicity as a function of dose

Dose (μg)	Total	No. of patients		
		Fever	Pain	Increased effusion
150	3	3	2	2
300	6	3	2	4
600	7	0	3	1
750	5	2	2	1
1000	4	1	1	0
1500	10	5	5	3
3000	5	2	0	0
Total (%)	40	16 (40)	15 (37.5)	11 (27.5)

reported in these tables was an episode of hepatitis and pneumonitis which appeared in one patient receiving 3000 μg for malignant ascites. The patient had a prior history of tuberculosis and had achieved a CR. The clinical course of this patient has been reported in more detail elsewhere (6).

DISCUSSION

This was a study of the efficacy and toxic effects of the administration of a nonviable mycobacterial vaccine into malignant effusions in patients with advanced malignancy. The primary toxic effects, fever and pain, were similar to those seen with conventional agents. A therapeutic effect manifested by the failure of effusions to reappear was seen in 44% of the patients. Response correlated with prior exposure to BCG vaccine or tuberculosis and with a febrile reaction following vaccine administration. It is possible that the immunologic memory for mycobacterial antigens results in an increased inflammatory response causing a greater sclerosis. An apparently higher CR rate seen in patients with pleural effusions may have been related to the limitation of the clinical examination in detecting residual ascites. This may have resulted in calling a patient a CR when a more sophisticated technique, such as ultrasound, would have revealed residual fluid and changed the response to a PR.

It is possible that some of our responses were due to the effects of systemic chemotherapy or hormonal therapy. There are three arguments which make this possibility unlikely: (*a*) because of our admission criteria, this conclusion would require a mixed and delayed response to phase II chemotherapy, ie, the effusion would have had to respond to systemic therapy while other measurable disease did not and would have to have been manifest only after no response to the first course; (*b*) among the patients not receiving chemotherapy, there was a response rate similar to that of the whole group; and (*c*) the correlation of response with prior BCG and fever suggests a cause-and-effect relationship between vaccine administration and response.

Optimal therapy of pleural effusion probably requires chest-tube drainage, a procedure which usually entails hospitalization and which was not used in this study. This may, in part, account for the relative low response rate seen in this study, relative to that of other agents such as tetracycline (7–9), where response rates in the range of 80%–100% have been reported. Determination of the exact activity of this vaccine will require a concurrent comparison using optimal techniques in homogenous patients.

Optimal therapy of peritoneal effusions is not as well-understood. Large volumes may be helpful in distributing the agent over the large surface area

TABLE 5.—Response according to site of effusion

Response	Site of effusion	
	Pleural (22 patients)	Peritoneal (12 patients)
CR	3 (13.6)	4 (33.3)
PR	7 (31.8)	1 (8.3)
Total	10 (45.4)	5 (41.6)

TABLE 6.—Toxicity according to site of effusion

Toxic effect	Site of effusion	
	Pleural (26 patients)	Peritoneal (14 patients)
Fever	12 (46)	4 (29)
Pain	12 (46)	3 (21)
Increased effusion	8 (31)	3 (21)
Gastrointestinal	—	7 (50)

(10,11). *Corynebacterium parvum* (12) and bleomycin (13) have been reported to be active in the treatment of malignant ascites. The Leveen shunt is an alternative to instilling therapeutic agents into the malignant effusion (14).

Although the treatment of effusions is often considered a palliative maneuver, it has been pointed out (15) that, in the later stages of the disease, certain patient groups, particularly those with breast carcinoma, have prolonged survivals after the appearance of an effusion. It may be worthwhile to evolve the therapeutic strategies which utilize these agents in first-line regimens, rather than as late, purely palliative maneuvers.

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Intensive Chemotherapy with Daunorubicin, 5-Azacytidine, 6-Thioguanine, and Cytarabine (DATA) for the Blastic Transformation of Chronic Granulocytic Leukemia[1,2]

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SUMMARY

Thirty patients with blastic transformation of chronic granulocytic leukemia were treated with a combination of daunorubicin, 5-azacytidine, 6-thioguanine, and cytarabine. Hematopoietic toxic effects were substantial and eight of 23 patients developed hepatic dysfunction (bilirubin > 5.5 mg/dl). Although the regimen resulted in marked decrease in peripheral blood blast cells and produced marrow hypoplasia in most of the patients (20 of 23), there was little therapeutic benefit. Seven patients died while aplastic, nine had recurrence of blastic leukemia, and only four had partial improvement in marrow function.

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After blastic transformation, chronic granulocytic leukemia (BT-CGL) is usually refractory to therapy, and the median survival of patients who enter this phase of the disease is usually only a few months. A combination of daunorubicin, 5-azacytidine, 6-thioguanine, and cytarabine (DATA) had produced a high frequency of remissions in a group of 14 patients with refractory adult acute myeloblastic leukemia (AML). For this reason, this same combination was studied in patients with BT-CGL and initially led to several favorable responses. Based on this experience, the use of this combination in BT-CGL was expanded to a groupwide phase II study by the Southeastern Cancer Study Group.

MATERIALS AND METHODS

Thirty patients with BT-CGL were entered in the study from April 1977 to March 1978. There were 17 males and 13 females, ranging in age from 18 to 72 years (mean, 45). Twelve patients had received prior treatment with vincristine and prednisone; three of these had achieved partial responses and then relapsed. All patients had > 30% blast cells in the bone marrow, with the exception of one patient who had biopsy-proven extramedullary blast cell crisis. None of the patients had received prior therapy with any of the agents in the DATA regimen. Seven patients were either asymptomatic or had mild symptoms of anemia, 13 had more severe symptoms, and ten were more seriously ill.

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Treatment

Each course of therapy consisted of daunorubicin iv (10 mg/m²/day for 5 days); cytarabine iv and 6-thioguanine orally (75 mg/m² of each every 12 hours for ten doses); and 5-azacytidine by constant iv infusion (150 mg/m²/day for 5 days). 5-Azacytidine was freshly prepared and administered in Ringer's lactate every 8 hours. Bone marrow biopsy was performed 2–5 days after each course of therapy, and if the marrow was not hypocellular a subsequent course of DATA was started. Treatment failure was defined as the inability to achieve a complete or partial remission after one episode of marrow hypoplasia or 2 months of therapy.

Evaluation of Toxicity

Cbcs were usually obtained daily (a minimum of three times a week), and a marrow examination was required prior to each course of therapy if leukemic cells had been cleared from the blood. Weekly BUN, serum creatinine, SGOT, bilirubin, and alkaline phosphatase were also obtained.

Criteria for Evaluability and Remission

Antileukemic effect, as determined by a decrease in the leukemic cells in the peripheral blood, was considered evaluable in patients who initially had > 1000 circulating blast cells and promyelocytes per microliter and who survived for > 10 days in the study. Patients were considered evaluable for marrow aplasia if the pretreatment marrow was hypercellular and if post-therapy marrow hypocellularity (< 15% cellularity) was documented by marrow biopsy. Patients who received one complete course of therapy and survived for at least 10 days in the study were considered evaluable for toxic effects.

Partial remission was defined as < 30% blast cells plus promyelocytes in the marrow, associated with peripheral blood neutrophil counts > 1000/μl, with or without peripheral blood platelet counts of > 50,000/μl. Complete remission was defined as < 10% blast cells plus promyelocytes in the bone marrow, associated with peripheral blood neutrophil counts of > 2000/μl and a platelet count of > 100,000/μl.

RESULTS

Therapeutic Effects

Prior to therapy, 21 of the 30 patients entered had circulating peripheral blood blast cell and promyelocyte counts > 1000/μl (mean, 47,000/μl; range, 1600–205,000). All of these patients achieved a peripheral blood blast cell plus promyelocyte count of < 100/μl. This required one to two courses (mean, 1.3) and, on the average, it first occurred 10.4 days after chemotherapy with DATA was started (range, 3–20 days).

Twenty-three patients had sufficient marrow examinations following treatment to determine antileukemic effect on the blast cells and promyelocytes in the marrow. Three of these patients failed to achieve hypoplastic marrows after two to three courses and survived 20, 34, and 43 days. Twenty of these patients achieved hypocellular marrows after one to three courses of therapy (mean, 1.8 courses). This was documented by a marrow biopsy performed from 10 to 61 days after starting therapy (mean, 23.4 days). Seven of these patients died while still aplastic (survival, 17–122 days; median, 38), and nine had recurrence of leukemic infiltrates in the marrow (survival, 33–230 days; median, 96).

The seven patients who had inadequate numbers of marrow examinations to determine the effect of the DATA regimen on the leukemic population in the marrow died relatively early during therapy (10–29 days; median, 18).

The median survival of all patients was 39 days from start of therapy.

There were no complete remissions; however, four patients did achieve partial marrow function in association with < 30% blast cells and promyelocytes in the marrow. The performance status was somewhat better than average in three of these responders. After marrow aplasia had been induced by two to three courses of DATA, granulopoiesis recovered in all four patients (peripheral blood neutrophil count > 1000/μl) and thrombopoiesis recovered in one patient (platelet count > 50,000/μl). One patient died of an apparent intracranial hemorrhage 2 days following recovery of granulopoiesis. The other three patients required no further therapy for 51, 65, and 102 days and survived 174, 184, and 236 days, respectively. Of these patients, two had had prior treatment with vincristine and prednisone and one had responded.

Toxicity

The adverse effect of the combination of DATA on residual pretreatment granulopoiesis and thrombopoiesis was substantial. Twenty patients had pretreatment peripheral neutrophil counts > 1000/μl and all experienced life-threatening neutropenia with associated fevers and infections. All 17 patients with pretreatment platelet counts > 50,000/μl required platelet transfusion support after therapy.

Development of abnormal hepatic and renal func-

tions during DATA therapy was relatively common. Bilirubin elevation was the most consistent laboratory abnormality pertaining to hepatic dysfunction. Of 23 patients with sufficient pre- and post-treatment bilirubin determinations, 15 had elevations to > 2 mg/dl (range, 2.1–16; mean, 6.9). Only one of these patients had an abnormal pretreatment bilirubin. In the eight patients with the highest bilirubin elevations (≥ 5.5 mg/dl), seven of the elevations occurred during the first 2–4 weeks of treatment, and the peak values in seven cases were related to death within 2–3 days.

Twenty-six patients had sufficient pre- and post-treatment BUN and serum creatinine determinations to assess renal toxic effects. On eight occasions, serum creatinine increased to > 2 mg/dl (maximum, 7.3 mg/dl), and/or the BUN increased to > 50 mg/dl (maximum, 120 mg/dl). All but one of these patients had normal pretreatment values. All of the patients were receiving cephalosporins and/or aminoglycosides at the time renal toxic effects were noted, so the direct relationship of renal dysfunction to DATA chemotherapy is uncertain.

All patients experienced mild-to-severe nausea and vomiting.

DISCUSSION

With continued progress in the treatment of adult acute leukemias, CGL is at risk of becoming the adult leukemia with the worst prognosis. The median survival of patients with CGL has remained constant over the past three decades at 39 months (1), related primarily to an inability to successfully manage these patients once they have undergone blastic transformation or metamorphosis to the more malignant phase of their disease.

This phase II trial by the Southeastern Cancer Study Group was conducted in an attempt to confirm the initial observation of three favorable responses to DATA therapy in the first three patients with BT-CGL treated at a single institution. The strategy of the DATA regimen was to give maximum tolerated doses of four agents, each with known activity in adult AML, to rapidly achieve marrow hypoplasia. This goal was achieved to a large extent in two thirds of the patients. The overall therapeutic effect of this aggressive approach, however, was negligible. Only four of the 20 patients whose marrows became hypoplastic had evidence of return of any normal hematopoiesis, but this was incomplete and transient. Most of the patients whose marrows became hypoplastic died in that state or had regeneration of blast cells in the marrow. Other trials using agents effective in treating AML have had similar disappointing results (2–4).

At present, there is a paucity of established, effective therapeutic regimens for the treatment of BT-CGL. A subgroup of these patients does respond transiently to vincristine and prednisone, and this set of patients can be identified to a large extent by lymphoblastic morphology (5), hypodiploidy (6), or an elevated terminal deoxynucleotidyl transferase (7). Other than the vincristine and prednisone regimen, the only combination that has shown promise is the eight-drug combination of 6-thioguanine, daunorubicin, cytarabine, methotrexate, prednisone, cyclophosphamide, vincristine, and L-asparaginase (TRAMPCOL) (8). The initial results with this combination yielded eight good responses among 19 patients; however, a larger study to confirm this result has yet to be conducted. With rare exceptions, all favorable responses to either vincristine and prednisone or AML-type regimens have been short-lived; 12-month remissions have been very unusual.

Two factors may account for the lack of success of aggressive chemotherapy designed to produce aplasia in BT-CGL. First, the kinetics may be unfavorable, with abnormal blast cells capable of regenerating at a rate similar to that of hematopoietic cells of the chronic phase of CGL (9). Second, there may be a paucity of chronic-phase stem cells remaining after aggressive chemotherapy either because of the natural course of the disease or the sensitivity of these cells to the chemotherapeutic agents. Autografting with stem cells cryopreserved during the chronic phase of CGL may provide a means of circumventing these problems by permitting more complete eradication of the leukemic blast cells with supralethal chemotherapy and/or radiation therapy and providing ample numbers of chronic-phase stem cells for marrow regeneration. Initial results with this approach appear encouraging (10) and worthy of further study.

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Totally Implanted Drug Delivery System for Hepatic Arterial Chemotherapy[1,2]

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SUMMARY

A totally implanted drug delivery system for hepatic arterial chemotherapy was evaluated in 13 patients with metastatic (11 colon and one carcinoid) or primary (one hepatoma) cancer of the liver. During laparotomy, a Silastic catheter was positioned in the hepatic artery for infusion to the entire liver arterial vasculature as ascertained by low-flow radionuclide angiography with ^{99m}Tc-macroaggregated albumin. The catheter was connected to a subcutaneously implanted model 400 Infusaid pump (Metal Bellows Corp, Sharon, MA). Each pump had a 50-ml volume and a set rate (3–6 ml/day) and required refill every 8–16 days. A side port bypassed the pumping mechanism and allowed direct catheter injection for nuclide angiography, for bolus drug administration, or for clearing of a blocked catheter. Pump refills and side port injections were performed by percutaneous injection. The 13 patients in this ongoing study received a median of 6 months (range, 4.5–17) of continuous hepatic arterial infusion. The pump performed reliably with stable (± 10%) flow rates and only one malfunction in 2800 cumulative days of use. Flow distribution determined by low-flow radionuclide angiography did not change in 12 patients. Patient acceptance was excellent, with the ability to participate fully in normal daily activities. Eleven patients showed partial hepatic tumor regressions documented by physical examination and nuclide liver scans. All patients were treated with 5-fluorodeoxyuridine. Two patients failed 5-fluorodeoxyuridine therapy and subsequently responded briefly to dichloromethotrexate. This implanted system should facilitate future investigation of regional chemotherapy using these and other agents.

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Since hepatic tumors derive their blood supply primarily from the hepatic artery, direct infusion of chemotherapeutic agents into the hepatic artery can potentially expose the tumor to much higher concentrations than are possible with conventional iv infusions and may lead to improved therapeutic effects. There are two crucial aspects to effective arterial chemotherapy. First, the chemotherapeutic agent utilized should have appropriate pharmacologic properties, ie, sufficient hepatic extraction and/or a short serum half-life (1–3). Second, the drug has to be reliably delivered into the arterial supply of the tumor-bearing liver; difficulties with the drug delivery system have limited the usefulness, applicability, and evaluation of hepatic arterial chemotherapy (4–8).

This study attempts to surmount the difficulties with prior systems through use of a nonthrombo-

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genic catheter surgically placed to reliably infuse the entire liver and through use of a reliable and convenient subcutaneously implanted pump.

MATERIALS AND METHODS

Patients

Thirteen adults (nine men and four women) with histologically proven, measurable primary or metastatic liver cancer (table 1) meeting the following criteria were entered in the study: vascular anatomy allowing complete (> 90%) infusion of the entire liver; hepatic tumor involvement as the primary factor in morbidity/survival; anticipated 2-month survival; recovery from prior surgery, radiotherapy, and chemotherapy; ability to tolerate surgical placement of the system; ambulatory at least 50% of the time; no significant heart, peripheral vascular, or renal disease; and a granulocyte count > 1500/mm^3, a platelet count > 100,000/mm^3, and a hematocrit level > 30%. Written informed consent was obtained from all patients.

Catheter Placement and Position

The hepatic arterial blood supply was defined by a preoperative contrast angiogram prior to laparotomy for catheter placement. Three patients with colon carcinoma had resection of the primary tumor plus catheter placement during one laparotomy without additional morbidity. The gastroduodenal artery was used for insertion of a Silastic catheter (0.092 inch in outside diameter × 0.015 inch in inside diameter; Metal Bellows Corp, Sharon, MA) into the common hepatic artery proximal to the right and left hepatic arteries whenever possible. In two patients with an aberrant left hepatic artery coming off the left gastric artery, complete hepatic infusion was achieved by catheter insertion into the common hepatic artery through the splenic artery and by tying off the branch of the left gastric artery to the stomach. In one patient with the right hepatic artery derived from the superior mesenteric artery, catheter insertion was accomplished by direct arteriotomy into the side of the right hepatic artery; subsequent nuclide flow studies showed infusion to 90% of the liver, although part of the left lobe was not covered by this one catheter. Catheter placement was successful in all patients after preoperative evaluation showed them to be suitable for the implantable system.

Hepatic infusion imaging was performed with 1 millicurie of ^{99m}Tc-macroaggregated serum albumin (Tc-MAA) injected at 0.5–1.0 ml/min into the catheter to duplicate the infusion rate and pattern of the chemotherapeutic agents as closely as possible. If necessary, the catheter tip was repositioned to obtain complete coverage of the tumor-bearing liver as ascertained by comparison of the infusion study with the ^{99m}Tc-sulfur colloid liver scan. The small bore of the catheter precludes contrast angiography.

A generous loop of catheter was then brought out through a separate stab wound in the peritoneal wall, a one-way (Gilson) valve was attached, and the line was maintained on an IMED pump (Medical Electronics Co) at 10 ml/hour with 5% dextrose containing 1000 units of heparin/250 ml.

Seven to 10 days later, when the patient had recovered from the laparotomy and was eating and ambulatory, another radionuclide flow scan was obtained to assure good hepatic flow distribution. Then, under local anesthesia, a sterilized model 400

TABLE 1.—Hepatic tumor responses with implanted hepatic arterial drug delivery system

Patient No.	Age (yrs)	Primary cancer	Duration of treatment (mos)	Duration of response* (mos)
1	62	Colon	17	PR (17+, ongoing)
2	50	Carcinoid	11	PR (11+, ongoing)
3	70	Colon	7†	PR (4+, dead)
4	66	Colon	8	PR (8+, ongoing)
5	62	Colon	8	PR (8+, ongoing)
6	26	Colon	6	PR (6+, ongoing)
7	43	Colon	6	PR (6+, ongoing)
8	60	Colon	6	PR (6+, ongoing)
9	58	Colon	6	PR (6+, ongoing)
10	53	Colon	5	SD (3, dead)
11	62	Colon	5	SD (5+)
12	42	Colon	5	PR (4+, dead)
13	33	Hepatoma	4.5†	PR (4, dead)

*PR = partial response; SD = stable disease.
†Responded to DCMTX after failing 5-FUdR.

Infusaid pump (figs 1 and 2; Metal Bellows Corp) was implanted subcutaneously into a pocket created in the abdominal wall. A sterile segment of catheter coming from the hepatic artery was connected in a subcutaneous tunnel to a similar Silastic catheter coming from the pump, using a barbed titanium connector (Metal Bellows Corp), thus totally implanting the system. Patients were ready for discharge 3–4 days after pump implantation, and the pump was filled with chemotherapeutic agent immediately prior to discharge. Figure 3 shows the completed system as implanted. (Recently, three additional patients not in the study have done well with a simultaneous catheter and pump implantation.)

Treatment Plan

The pump was filled with 5-fluorodeoxyuridine (5-FUdR) in heparinized saline at a concentration that resulted in delivery of 0.3 mg/kg/day of 5-FUdR and 1000 units/day of heparin. Although the flow rate for an individual pump was constant, the flow rates among the available pumps ranged from 3 to 6 ml/day. Since the pump reservoir contains 50 ml, refills were required every 8–16 days.

For pump refill, the area of the skin overlying the pump was cleaned with providone iodine, and a 22-gauge Huber-point needle (Vita Needle Co, Needham, MA) attached to an empty syringe barrel was inserted into the pump through the central septum. The residual volume was removed and recorded for calculation of flow rate. Without removing the needle, the pump was refilled with drug solution, using a 50-ml syringe. When diarrhea or other drug-related toxic effects occurred, the 5-FUdR was drained and the pump was maintained on heparinized saline alone until resolution of toxicity. 5-FUdR was then reinstituted at a 25%–50% dose reduction. When no toxicity was seen at a given dose and when the maximum tolerated dose had not been determined for an individual patient, the 5-FUdR dose was escalated (0.2 mg/kg/day) at monthly intervals. When progressive disease occurred despite achievement of a maximum tolerated dose, 5-FUdR was discontinued and dichlorometh-

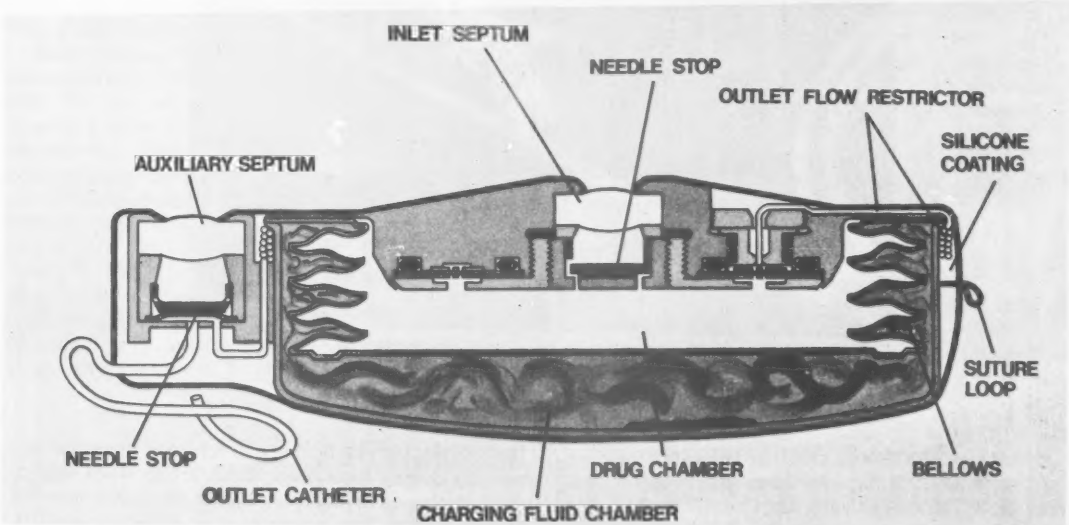

FIGURE 1.—Scheme of model 400 Infusaid pump produced by Metal Bellows Corp. This pump has a driving mechanism similar to that of an earlier model developed by Blackshear et al (18) for continuous heparin infusions and used without side port for a small study for hepatic arterial infusion (19). Model 400 has a side port with an auxiliary septum which bypasses the pumping mechanism for direct catheter injection. The disc-shaped pump is separated into 2 chambers by a titanium bellows. The inner drug chamber contains the solution to be infused; the outer charging chamber contains a fluorocarbon liquid in equilibrium with its vapor phase. At 37°C, the vapor pressure exerted by this substance is approximately 300 mm Hg greater than atmospheric pressure. This vapor pressure provides the power source, exerting pressure on the bellows and forcing infusate through a 0.22μ bacterial filter and a flow-regulating resistance element. The pump is placed beneath the skin, and the inner drug chamber is refilled by percutaneous injection through a self-sealing septum. The pressure of this injection condenses the driving power source, thus simultaneously filling the pump and recharging the power source. The pump has a 50-ml capacity, and flow rates of individual pumps are factory calibrated to deliver a constant set rate, as desired, in the range of 3–6 ml/day. Diagram courtesy of Metal Bellows Corp.

FIGURE 2.—Model 400 Infusaid pump weighs approximately 200 g and has overall dimensions of 3 × 9 cm. This photograph courtesy of Metal Bellows Corp.

otrexate (DCMTX) was instituted at a starting dose of 2 mg/m²/day. Multiple determinations of 5-FUdR and DCMTX levels (using high-performance liquid chromatographic assays [1]) showed that there was no breakdown of either drug in the implanted pumps. To prevent gastritis and associated bleeding, patients received 300 mg of oral cimetidine four times a day after start of chemotherapy (4,9).

A partial response was defined as at least a 30% decrease in the sum of the palpable liver size below the costal margin in the right midclavicular line and below the xiphoid process. In addition, all responders showed a 50% reduction in the product of the longest perpendicular diameters of measurable nodules during liver scan. CEA levels were originally increased in all patients with colorectal cancer and decreasing levels correlated in all instances with the response determined by physical examination and liver scan. ^{99m}Tc-sulfur colloid liver scans were obtained every 2 months.

RESULTS

The 13 patients in this study have received a median of 6 months (range, 4.5–17) of continuous hepatic arterial drug infusion (table 1). After the

10–14 days in the hospital required for system implantation, patients have received all therapy as outpatients and five have had pump refills by their referring physicians. All patients readily tolerated implantation and maintenance of the drug delivery system and were able to return to a level of activity greater than or equal to that achievable before surgery. Five of the patients have continued to work in their full-time occupations.

The limiting toxic effects of continuous hepatic arterial infusion of 5-FUdR were gastrointestinal: anorexia, epigastric distress (heartburn), abdominal cramps, and diarrhea. Mild elevations in serum bilirubin and in SGOT were detected in most patients after 6–8 weeks of continuous drug infusion. All gastrointestinal symptoms and signs of chemical hepatitis resolved when 5-FUdR was withheld for 1–4 weeks. Recently, drug-free "break periods" have been instituted with every other refill and appear to prevent such toxicity. No patient developed leukopenia or thrombocytopenia while receiving 5-FUdR. The two patients receiving DCMTX (table 1) had toxic effects similar to those with 5-FUdR except that, in addition, one patient developed mucositis and thrombocytopenia, which resolved with 10 mg/day of oral leucovorin calcium.

Table 2 shows the relative lack of complications encountered in 2800 days of infusion with the system. The two instances of catheter occlusion were readily cleared by direct flushing of the catheter through the side port. There have been no infectious or bleeding complications. In one patient the radionuclide flow study changed after 3 months to show infusion to about 30% of the liver, whereas the entire hepatic arterial vasculature was originally infused. In the other 12 patients, radionuclide flow distribution has not changed during infusion, essentially ruling out perforation of the hepatic artery and significant arterial thrombosis. Contrast angiography was performed in five patients 3–6 months after system implant and showed no arterial thrombosis in four patients. However, there appeared to be partial hepatic arterial thrombosis proximal to the catheter tip in the fifth patient. Figure 4 shows a Tc-MAA flow study performed through the side port 8 months after implantation of the system, demonstrating complete infusion to the entire liver. One pump malfunction occurred after 4 months of treatment in patient 10 due to an occlusion in the outlet flow restrictor (fig 1). Pump flow rates have been remarkably constant (table 3).

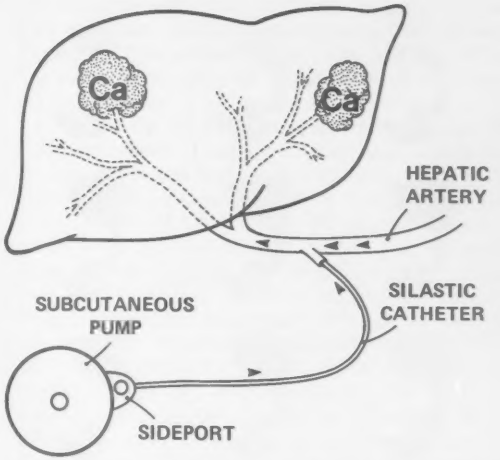

FIGURE 3.—Diagram of the totally implanted drug delivery system with catheter inserted so as to feed drug to entire (tumor-bearing) liver.

Eleven of the 13 patients (85%) in the study achieved partial response of hepatic tumor. Initial evidence of hepatic tumor response was noted by 3 weeks after start of treatment in most patients and improvement has been ongoing (table 1). Four

TABLE 2.—Complications of implanted hepatic arterial drug delivery system in 13 patients

Complication	No. of occurrences	Time after implant	Comments
Catheter occlusion	2	1 wk, 2 mos	Cleared with side port flush
Arterial perforation and thrombosis	0		
Flow change distribution	1	3 mos	After response
Pump malfunction	1	3 mos	Irreversible
Bleeding	0		
Infection	0		

*2800 total days of infusion for group; median of 180 days (range, 130–510) for individual patients in group.

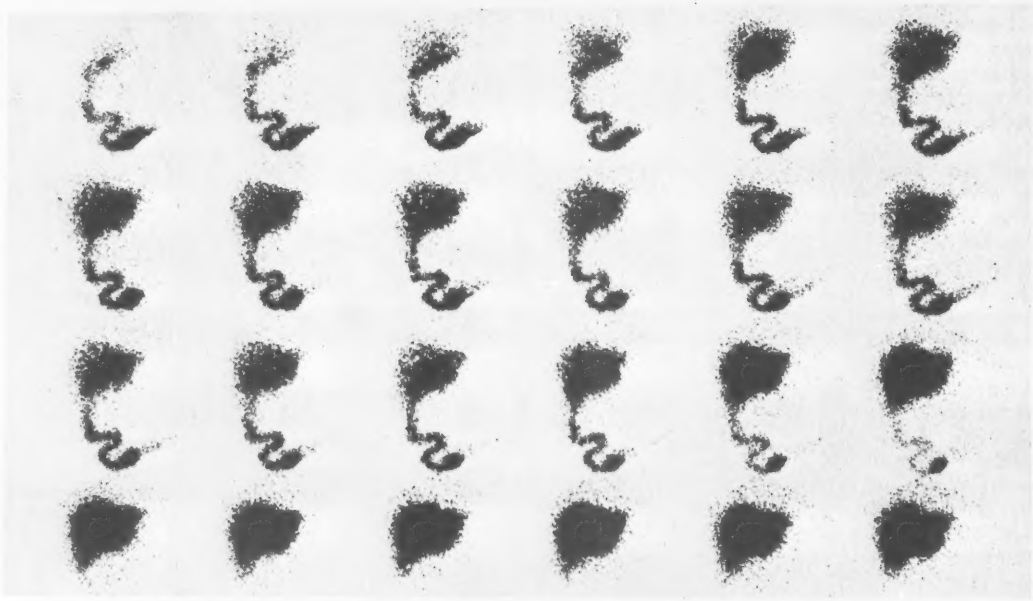

FIGURE 4.—Low-flow radionuclide angiography performed through the implanted pump side port using Tc-MAA (*see* text). These sequential frames (starting in the left upper corner and ending in the lower right corner) were taken at 2-sec intervals. The initial frames demonstrate catheter filling, with eventual complete infusion to the entire liver, confirmed by matching of the infusion scan pattern to a ^{99m}Tc-sulfur colloid scan pattern.

patients developed rapidly progressive and fatal extrahepatic disease while the tumor in the liver was still controlled. The median response duration and survival for this group of patients has not been reached.

DISCUSSION

Hepatic arterial drug infusions have been used therapeutically for 20 years. Although 5-FUdR is the most rational of the two agents for hepatic arterial infusion (1), the results reported with 5-FU or 5-FUdR given by hepatic arterial infusions have often been superior to those achieved with 5-FU given by the usual iv route (5,7,10,11). However, a recently reported Central Oncology Group study demonstrated that a 21-day induction regimen of hepatic arterial 5-FU followed by maintenance weekly iv 5-FU does not necessarily generate a statistically improved response rate over iv 5-FU alone

TABLE 3.—Pump flow rate variability in vivo

Pump (serial No.)	Duration of infusion (mos)	No. of refills*	Daily rate (ml)†
001	5.0	11	3.80 ± 0.14
002	2.5	10	4.26 ± 0.19
004	4.0	13	3.82 ± 0.23
109	3.5	12	5.18 ± 0.19
264	2.5	7	5.50 ± 0.28
266	3.5	10	3.73 ± 0.27
268	3.5	10	3.52 ± 0.23

*Refilled every 7–14 days.
†Values = mean ± SE.

(12). Unfortunately, there are insufficient data given in this multi-institutional study to judge quality control. It is obvious that the response rate in hepatic arterial drug infusions will depend critically on how reliably the drug is delivered to the entire tumor-bearing liver. Whereas iv drug administration can be done reliably, the odds are against the reliable administration of hepatic arterial chemotherapy due to the multiple potential problems in drug delivery. The unique problems of hepatic arterial infusion include pump malfunction, catheter blockage or disruption, arterial thrombosis and/or perforation, infection, and bleeding (4,9). The high (often unrecognized) incidence of hepatic arterial thrombosis with hepatic arterial infusion therapy has raised the question as to the role of thrombosis-induced tumor ischemia in therapeutic response (5). Any potential therapeutic effect of catheter-induced hepatic arterial thrombosis compromises the evaluation of drug effect. The development of collaterals after thrombosis (13) often makes further arterial drug delivery impossible since tumors become supplied by a multiplicity of arterial sources.

Pumps used previously have required long-term hospitalization or the wearing of a bulky and inconvenient pump/harness. The difficulties of reliably administering intra-arterial therapy to outpatients, even with a technically advanced external pump, remain formidable (14). Despite its recent introduction, the implanted titanium model 400 Infusaid pump used in this study appears to overcome most of the previous difficulties of outpatient pumping systems. Flow rate variation in vivo was low (± 10%), and there was only one pump malfunction in 13 pumps used, for a cumulative total (to date) of > 2800 days of continuous arterial infusions. The implanted pump did not interfere with the usual daily activities of patients and five of the 13 patients returned to work. There was no difficulty in refilling the pump and five patients have received most refills from their referring physicians. Although the flow rates on several of the pumps were such that refill was required every 9–10 days, the latter pumps used in this series required refill only on a 14-day basis.

The pump side port which connects directly into the catheter can serve three purposes. First, should flow through the catheter become obstructed by blood clot (such as is likely to occur if the pump is allowed to run dry), side port injection can be used to unplug the catheter. Second, the side port allows the use of Tc-MAA for low-flow radionuclide angiography to monitor drug flow distribution (and changes in tumor vascularity). Finally, direct side port injections can be used to administer other drug solutions that are best given at different rates, that are unstable with time, or that are incompatible with exposure to the central pump mechanism. Therefore, this system is versatile and will allow the use of intra-arterial drugs alone or in combination, as bolus or as continuous infusions.

Catheter tip position in the arterial tree is of utmost importance. If drug flow distribution does not go directly to the tumor capillary bed, there is no reason to expect selectivity or tumor response. Low-flow (Tc-MAA) radionuclide angiography appears to be the best method currently available to measure flow distribution. One example is patient 8 who had the Silastic catheter implanted at another hospital. Nuclide angiography showed flow only to the left lobe. A pump was implanted nonetheless, and after 2 months of treatment the tumor in the right lobe had grown dramatically. The catheter was surgically replaced to infuse the entire liver. After 2 months of infusion to the entire liver, the liver scan, physical examination, and overall clinical state all revealed good response. Although repeat nuclide flow studies showed stable infusion to the entire liver in 12 patients, in one patient who had dramatic tumor and liver shrinkage, the pattern changed after 3 months to cover only the left lobe. Since this patient's response has been maintained, we have not yet investigated catheter position and the arterial status with percutaneous contrast angiography.

We have focused on the drug delivery system in this study since this element must be reliable for all hepatic arterial drug testing. Certainly, the system we have described requires considerable initial effort due to the surgery involved. Furthermore, patients who can tolerate surgery would be expected to have less advanced disease and thus a longer projected survival without any effective treatment. Maximizing the response rate should lead, barring lethal toxicity, to the longest survival times. However, although the liver tumor may be originally the leading source of morbidity, once liver disease becomes controlled, tumor outside the liver may become the primary determinant of morbidity and death, as was the case in all four of the patients who have died. Therefore, the patients who are most likely to reap the greatest benefit from this form of treatment are those with tumor confined to the liver. The high response rate (85%, 11 responses among 13 patients) in this study is gratifying, and even with this small number of patients it suggests that a true response rate > 50% is statistically probable (15).

Although this study has focused on the use of this drug delivery system for hepatic arterial chemotherapy, the same system should prove beneficial for intra-arterial infusions in other body regions,

such as those for head and neck cancer (16) and brain tumors (17). Inasmuch as the same system will readily give iv infusions, at some future time, randomized controlled studies of iv versus intra-arterial infusions will be indicated.

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Implanted System for Intraventricular Drug Infusion in Central Nervous System Tumors[1,2]

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SUMMARY

We have developed a totally implanted drug delivery system capable of maintaining constant cerebrospinal fluid (CSF) drug levels through continuous intraventricular infusion in outpatients. This system was used to infuse methotrexate (MTX) intraventricularly in seven patients with incurable CNS malignancies. One patient with meningeal diffuse histiocytic lymphoma, five patients with grade III–IV astrocytomas, and one patient with melanoma metastatic to the brain were treated with this system for 4–40+ weeks. The system consists of an Infusaid pump (Metal Bellows Corp, Sharon, MA), implanted subcutaneously in the infraclavicular fossa, which delivers a drug-containing solution at a set rate (3–5 ml/day) through a subcutaneous silastic catheter to a Rickham ventriculostomy reservoir and into a lateral ventricle. System placement and maintenance were readily tolerated. Constant MTX infusion at rates of 0.5–10 mg/day generated corresponding constant CSF drug levels in the range of 2–30 μM. Simultaneous serum MTX levels were undetectable (< 0.01 μM), indicative of a 200- to 3000-fold selective regional concentration advantage for this approach. CNS toxic effects included transient meningism and fever (four patients), transverse myelitis (one), and the development of a diffuse hypodensity of the white matter on computerized tomographic scan which was not associated with any neurologic deficit (two). The usual systemic toxic effects (myelosuppression and mucositis) of MTX were not seen. The patient with meningeal lymphoma has had a complete remission of meningeal disease continuing past 10 months. Computerized tomography showed that three of the five high-grade astrocytomas had 25% size reductions in tumors lasting 2–6 months. This system may provide a means for improved treatment of meningeal tumor although its role in the treatment of intraparenchymal brain tumors is less clear. Of greater consequence, however, is the demonstrated ability of this system to maintain a controlled CSF drug level which should prove useful in many areas of therapeutic research.

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Neoplastic involvement of the CNS usually carries an ominous prognosis with survival measured in a few months (1–4). The blood-brain barrier which limits drug access to the cerebrospinal fluid (CSF)

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space and to brain parenchyma may play an important role in the lack of drug effectiveness against CNS neoplasia in that many systemically administered antineoplastic drugs penetrate the CSF or brain tissue poorly and do not achieve the therapeutic levels attained elsewhere in the body (5,6).

Direct intraventricular injection can produce high drug concentrations in the CSF (7). In addition, the restricted transport of drugs such as methotrexate (MTX) and cytarabine (Ara-C) between the CSF space and the systemic circulation, in conjunction with a much smaller volume of distribution in the CSF as compared to the systemic circulation, makes it possible using direct intraventricular injection to generate CSF drug concentrations that are 100- to 1000-fold greater than corresponding plasma drug levels (7). With this differential the higher CSF drug concentration may be well within the therapeutic range while the much lower, systemic levels are nontoxic. Intraventricular drug therapy using MTX and Ara-C has been shown to be effective treatment for overt meningeal leukemia and lymphoma (2,3,8–10). Intraventricular drug therapy for meningeal involvement by solid tumors has been less successful, possibly due to greater intrinsic resistance which would require greater drug exposure for effective therapy.

It seems reasonable to postulate that the maintenance of a controlled, constant CSF level of drug would have the potential of decreasing systemic toxicity by avoidance of high peak plasma levels and of increasing the cytotoxic effects against tumor cells in the CSF by the elimination of low, nontherapeutic trough levels. Maintenance of the highest CSF drug level possible should also maximize the driving force for diffusion of drug from the CSF space into adjacent brain (parenchymal) extracellular fluid space.

The recent clinical availability of a totally implantable infusion pump made it possible to attempt constant intraventricular drug infusions with decreased risk of infection. The compatibility of the pump with MTX permitted a pilot clinical pharmacologic study of constant intraventricular MTX infusion in patients with meningeal and intraparenchymal brain malignancies. The objectives of the study were (*a*) to evaluate the reliability, safety, and convenience of the drug delivery system, (*b*) to determine the regionally selective drug levels and toxic effects of constant intraventricular MTX infusion, and (*c*) to ascertain in a preliminary way the therapeutic potential of this treatment modality for CNS neoplasia.

METHODS

Patients

The study (table 1) included seven patients with histologically proven CNS malignancy (one patient with meningeal involvement from diffuse histiocytic lymphoma, five with grade III–IV astrocytomas, and one with malignant melanoma metastatic to the brain). There were four women and three men with a median age of 46 years (range, 34–62). No patient had received prior cranial irradiation. Written, informed consent was obtained from all patients.

Drug Delivery System

The totally implantable drug delivery system consists of a subcutaneously placed model 100 Infusaid

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Diagnosis	Location	Symptoms	MTX dose (mg/day)	Duration of infusion (wks)	Response
1	62	Diffuse histiocytic lymphoma	Leptomeninges	Right leg weakness	0.5*	40* (ongoing)	Complete
2	34	Astrocytoma, grade III	Right temporo-frontal lobe	Left hemiparesis, hemianopia, headache	2–6	6	None
3	45	Metastatic melanoma	Bilateral multiple metastasis	Abnormal behavior, left hemiparesis	2–10	6	None
4	41	Astrocytoma, grade III	Right parietal lobe	Seizures	0.5–8	12	Probable
5	37	Glioblastoma multiforme	Right lateral ventricle	Left hemiplegia	0.5–2	12	Probable
6	52	Glioblastoma multiforme	Right parietal lobe	Headache, left hemiparesis	0.5–2	12	Probable
7	52	Glioblastoma multiforme	Left occipito-parietal lobe	Hemianopia	0.25–0.5	4	None

*Received 6-day infusion every 4 wks.

pump (figs 1 and 2) (Metal Bellows Corp, Sharon, MA) connected to a fine-bore silastic catheter tunneled subcutaneously and connected to the side arm of a Rickham ventriculostomy reservoir (fig 3). The Rickham reservoir was selected because of its small (~ 0.5-ml) dead volume. The entire system (pump, catheter, and reservoir) can be implanted under genèral anesthesia at one sitting. However, initial reservoir placement separately provides assurance of normal ventricular outflow and of a functioning ventricular access prior to pump implantation. Thus, in all but one patient, the system was implanted in two stages, the first stage being placement of the ventriculostomy reservoir under local anesthesia. Prior to reservoir placement, a 5–6-cm piece of thick-wall, fine-bore (0.092 inch in outside diameter × 0.015 inch in inside diameter, 35-μl volume/foot) silastic catheter was attached to the side arm of the Rickham reservoir with medical adhesive (Silastic Medical Adhesive Silicone Type A, Dow Corning Corp, Midland, MI). The distal 1–2 mm of the catheter tip attached to the side arm was plugged with adhesive to prevent CSF leakage prior to connection to the pump. After 1–2 days, a radionuclide cisternogram using ytterbium 169-labeled diethyltriamine pentaacetic acid was performed through the ventricular reservoir to ascertain normal bulk flow of CSF. Criteria similar to those described by Dichiro et al (11) were used to define the normalcy of the bulk flow of CSF in our group of patients.

In the second stage, the titanium pump, which is approximately the size of a pacemaker, was implanted in a subcutaneous infraclavicular pocket. The silastic outflow catheter (of the same dimensions as that attached to the reservoir) was tunneled subcutaneously up the side of the neck, behind the ear, and under the scalp forward to the ventriculostomy reservoir. The silastic pump outflow catheter and reservoir side port catheter were then joined with a barbed titanium connector (obtained from Metal Bellows Corp). Although the pump can be implanted with local anesthesia, the tunneling of the catheter necessitates general anesthesia.

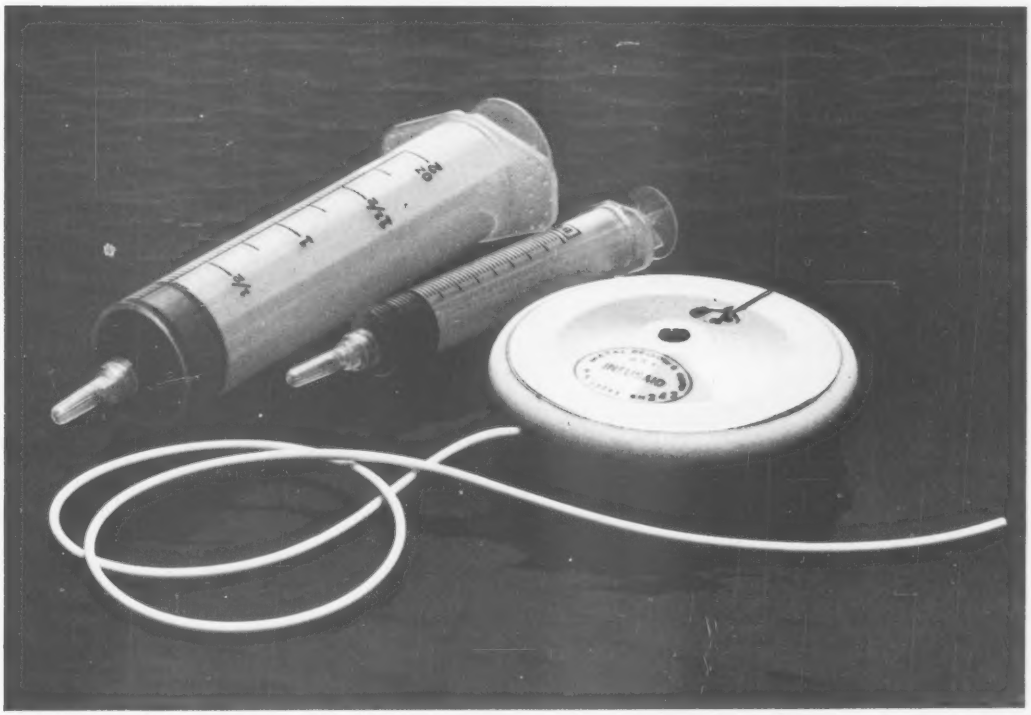

FIGURE 1.—Photograph of the model 100 Infusaid pump (courtesy of Metal Bellows Corp). The pump weighs 180 g and has a diameter of 9 cm and a thickness of 3 cm.

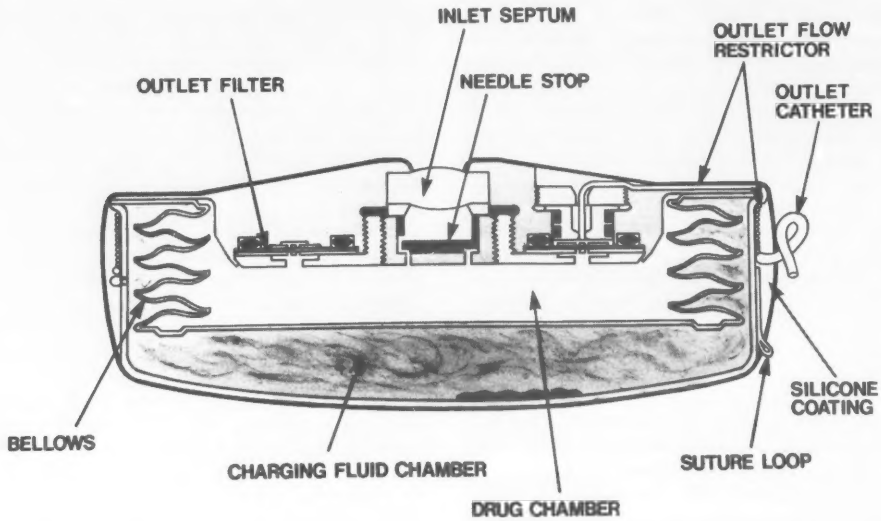

FIGURE 2.—Scheme of the model 100 Infusaid pump (courtesy of Metal Bellows Corp). The disc-shaped pump is separated into 2 chambers by a titanium bellows. The inner drug chamber contains the solution to be infused and the outer charging chamber contains a fluorocarbon liquid in equilibrium with its vapor phase. At 37°C, the vapor pressure exerted by this substance is approximately 300 mm Hg greater than atmospheric pressure. This vapor pressure provides the power source, exerting pressure on the bellows and forcing the infusate through a 0.22-μ bacterial filter and a flow-regulating resistance element. The pump is placed beneath the skin, and the inner drug chamber is refilled by percutaneous injection through a self-sealing septum. The pressure of this injection condenses the driving vapor, thus simultaneously filling the pump and recharging the power source. The device is coated with silicone rubber and the infusate is introduced into a vein, artery, or body cavity through a fine-bore silastic catheter. Each clinical pump has a 50-ml capacity. The flow rates of individual pumps are factory-calibrated to deliver a constant rate in the range of 1–6 ml/day.

The pump is refilled by insertion of a 22-gauge deflected (Huber) point needle (Vita Needle Co, Needham, MA) through the skin into the central rubber inlet septum (fig 2). The force of the injection into the expansible inner drug chamber compresses freon gas in the outside charging chamber into the liquid form. Reversal of this transformation of freon (ie, from the liquid back to the gaseous state) at body temperature provides the constant driving force that propels the drug solution through a resistance element and the catheter into the ventricle. Each pump has a factory-calibrated flow rate. In vitro at 37°C the daily flow rate varies < 5% from the mean (measured over at least 30 days). Each pump has its individual flow rate which ranged from 3 to 5 ml/day within the series of pumps used in this study. Since the model 100 pump has a 50-ml volume, refills were necessary every 10–14 days for a constant infusion. However, for intermittent short-term courses of continuous infusion, the volume of drug injected into the pump was calculated so that the pump would run dry at the end of the desired period of time.

Administration and Determination of MTX Levels

Preservative-free MTX was used in all but the initial patient. Elliott's B solution (obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) was used as the diluent for MTX throughout. In the initial three patients a starting dose rate of 6–10 mg/day was selected based on our prior experience with bolus intraventricular injection. However, the resulting CSF levels of MTX were extremely high and produced fever and mild meningism in the first three patients. Subsequent patients received initial dose rates of 0.5–2 mg/day. The patient with meningeal lymphoma has received 6-day courses of continuous infusion every 4 weeks. The amount of MTX formulated in the pharmacy for each refill was derived from the following calculation:

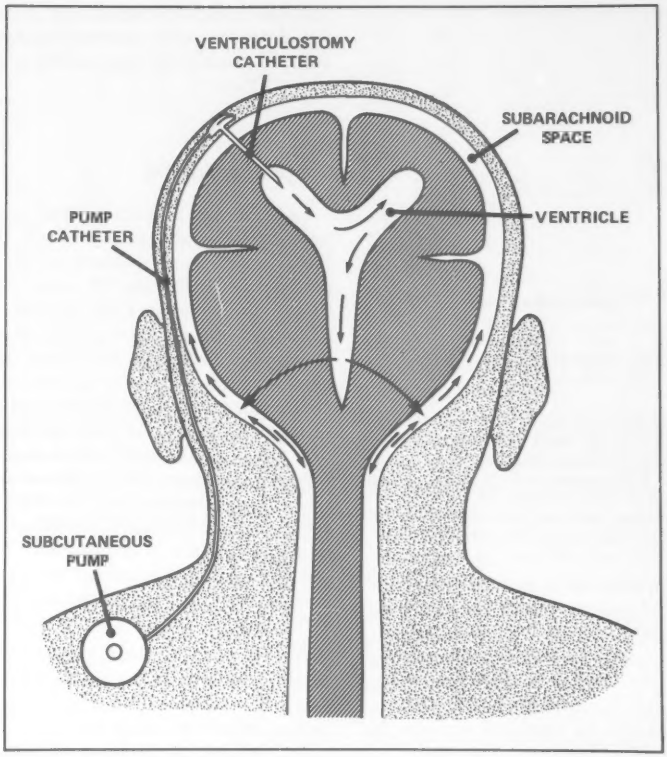

FIGURE 3.—Scheme of the totally implanted system for constant intraventricular drug infusions consisting of a subcutaneous Infusaid pump, silastic catheter, and ventriculostomy reservoir and catheter.

$$\text{amount of MTX for refill} = \frac{\text{"nominal"}^4 \text{ dose rate} \times \text{capacity of pump}}{\text{flow rate of pump in vitro}}.$$

After 7–14 days at each dose rate, CSF samples were obtained from the ventriculostomy reservoir for determination of MTX level. Pump infusate was cleared from the reservoir and ventriculostomy catheter by rapidly drawing six sequential samples of 1.5 ml each from the reservoir (fig 4). The MTX levels in the fifth and sixth samples were identical in every instance and were considered to represent the ventricular drug level. In two patients, we also determined MTX levels of the drug solution placed into and removed from the pump at the time of refill. In these two patients the "actual"[5] dose rate of MTX delivered to the ventricles was calculated and used for data analysis.

MTX levels were measured by HPLC using a Waters Associates liquid chromatograph equipped with a model 440 UV absorbance detector. An internal standard (aspartic acid derivative *N*-[2,4-diamino-6-quinazolinyl]methylaminobenzoyl, NSC-112846) was added to a final concentration of 5 μg/ml in all MTX-containing samples. A Waters Associates μBonda-pak C18 column was used in the analysis. The solvent system consisted of 40% methanol in 0.01 M phosphate buffer (pH 7.7) containing tetraheptylammoniumchloride (120 mg/liter). MTX concentration was determined using the peak height

[4]"Nominal" dose rate is defined as the product of the drug concentration formulated by the pharmacist multiplied by the factory-determined pump flow rate in vitro.

[5]"Actual" dose rate is defined as the product of the MTX concentration in the pump (determined by high-performance liquid chromatography [HPLC]) multiplied by the mean flow rate of the pump measured on a given refill cycle in vivo.

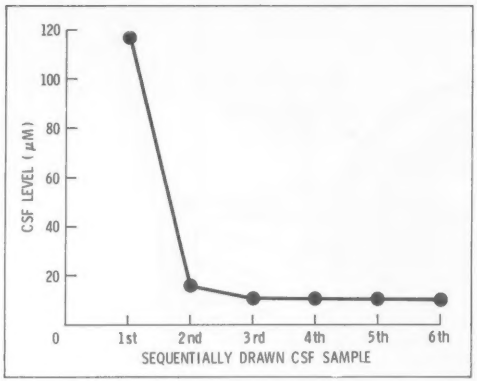

FIGURE 4.—Determination of ventricular CSF level of MTX as the asymptote achieved through rapid sequential sampling (1.5-ml samples) from the reservoir. The initial fall in level demonstrates clearing of MTX infusate from the reservoir and ventriculostomy catheter.

ratio method with the internal standard. All samples were measured in triplicate. MTX serum levels were measured by radioenzymatic assay (12) as the level in all instances was well below the sensitivity of the HPLC assay. The HPLC assay is sensitive to 0.5 μM and the radioenzymatic assay to 0.01 μM. Variability for both assays on replicate samples is within 10%.

Assessment of Pump Flow Rate

The preset flow rate of each pump was determined by the company during in vitro incubation of the pump at 37°C. To determine the in vivo flow rate the residual volume was measured each time the pump was refilled. The average pump flow rate was then calculated as a ratio of the infused volume over the time elapsed in days.

Monitoring

At each visit for a pump refill, ie, every 7–14 days, patients were examined and the following were determined: CSF pressure, protein, glucose, cell count, and differential; cbc with differential and platelet counts; serum glucose, BUN, and creatinine; and plasma and CSF MTX level. The SGOT, alkaline phosphatase, LDH, and bilirubin levels were determined at least monthly.

Computerized tomographic (CT) brain scans (with and without contrast enhancement) were obtained before implant (3–5 weeks after partial resection in the case of astrocytomas) and at least every 6 weeks for patients with intraparenchymal tumors and every 3 months for the patient with meningeal cancer.

Response Criteria

Criteria for response were adapted from those previously suggested by Levin et al (13). A complete response is defined as the complete disappearance of tumor on CT scan or clearing of malignant cells from the CSF (in the case of meningeal neoplasm) with total reversal of neurologic deficit. A probable response is defined by unchanged neurologic status, stable or decreased tumor size, or a reduced number of malignant cells in the CSF while doses of steroids are held constant. A nonresponse denotes clinical deterioration and increasing size of the tumor as seen by CT scan or an increasing number of malignant cells in the CSF. The need to increase steroid doses to stabilize neurologic status is indicative of a nonresponse.

Patients were followed closely and when it became clear that a given patient was not responding to intraventricular therapy, radiotherapy was initiated.

RESULTS

System Convenience and Reliability

There were no surgical, infectious, or neurologic complications related to the implantation and maintenance of the system. Refilling of the pump and sampling from the ventriculostomy reservoir were carried out without adverse sequelae. After the initial hospitalization of 5–7 days for the surgical implantation of the system, therapy was done exclusively on an outpatient basis. No patient requested removal of the system and the presence of the system did not impede subsequent cranial irradiation or systemic chemotherapy.

All pumps performed reliably for 4–40+ weeks. The mean pump flow rates determined over successive 7–14-day intervals for 3 months in three patients and 1½ months for a fourth patient correlate well with the mean flow rate determined in vitro prior to implant (fig 5).We found that it was possible to give intermittent cycles of infusional therapy since the infusion stops whenever the pump empties itself and since CSF does not generally clot to block the catheter. The system restarted reliably even when the pump was refilled weeks later.

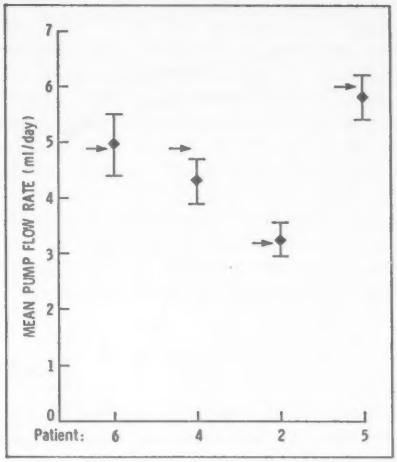

FIGURE 5.—Variability (mean ± 1 SE) in pump flow rates in 4 patients determined over 7–10-day refill intervals during a 2–3-mo period. Arrow = in vitro flow rate against atmospheric pressure as determined by the Metal Bellows Corp.

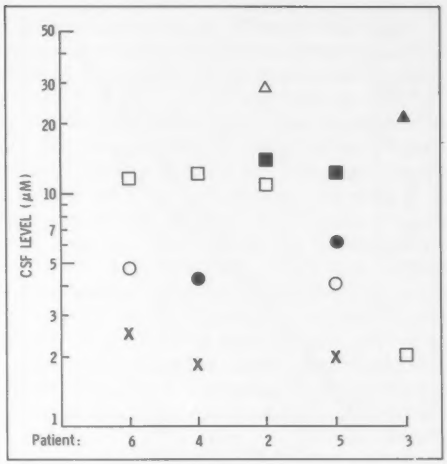

FIGURE 6.—CSF levels of MTX in 5 patients at progressively escalated nominal dose rates ranging from 0.5 to 10 mg/day. Symbols correspond to the following MTX dose rates in mg/day: × = 0.5, ○ = 1, ● = 1.5, □ = 2, ■ = 3, △ = 6, ▲ = 10.

MTX Levels

Although in vitro testing indicated that MTX did not react with the pump, in vivo drug stability studies were done on the first three patients. Comparison of the MTX level in the pump at the initiation of a refill cycle and 7–14 days later at the end of the cycle in all cases showed no difference (ie, levels were within 5% of each other). Furthermore, although the HPLC assay can detect the usual breakdown products of MTX, none were ever seen in the samples removed from the pumps or in the CSF.

The ventricular MTX levels ranged from 2 to 30 μM with nominal dose rates from 0.5 to 10 mg/day (fig 6). Five patients had intraventricular levels maintained at ≥ 2 μM for 6 weeks. In three of the five patients, levels were maintained at ≥ 2 μM for 3 months. Relatively comparable MTX levels were achieved in different patients at the same nominal dose rates regardless of their body surface area (fig 6). At a nominal dose rate of 2 mg/day, the intraventricular MTX level was 12 (± 2) μM in three patients. At an MTX dose rate of 0.5 mg/day, the CSF level ranged between 1.8 and 2.5 μM in four patients. CSF levels were somewhat lower at given dose rates in the patient with metastatic melanoma compared to the levels in other patients (fig 6; patient 3). Ventricular CSF levels of MTX appear to be a linear function of actual dose rate infused as shown in the two patients for whom actual dose rates were determined (fig 7). Relatively constant MTX levels of CSF were maintained throughout a given 7–14-day infusion cycle when the patient's clinical status was stable (fig 8). The drop in MTX level in patient 7 (fig 8) was temporally related to a marked increase in intracranial pressure and brain edema coincidental with rapid tumor progression.

Serum MTX Levels

Simultaneous serum and CSF MTX levels were determined in each patient. Serum MTX concentrations in all instances were < 0.01 μM, the lower limit of sensitivity of the radioenzymatic assay. As noted above, simultaneous CSF levels of MTX were between 2 and 30 μM. Hence, the ratio of the CSF level to the systemic MTX level indicates a sustained 200- to 3000-fold regionally selective exposure advantage in the CSF space.

Toxicity of MTX Treatment

There was no acute CNS toxicity associated with the removal of CSF samples using the reservoir. Toxicity related to the continuous intraventricular infusion of MTX is summarized in table 2. Three patients with astrocytomas developed fever and mild meningism within 24 hours of initiation of the MTX infusion. In one of these three patients fever did not recur on subsequent infusions. In the other two, fever (but not meningism) recurred and was readily suppressed with systemic corticosteroids.

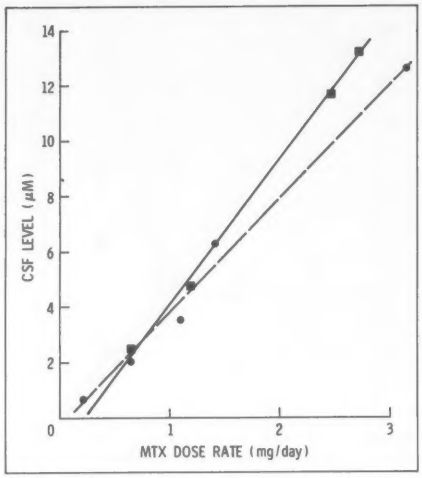

FIGURE 7.—CSF level of MTX as a function of actual dose rates in 2 patients. Lines were plotted by linear regression.

TABLE 2.—Toxicity observed

	No. of patients	MTX dose rate (mg/day)
Fever and meningism	4	0.5–6
Transverse myelitis	1	6
Diffuse hypodensity of white matter on CT scan	2	0.5–2
Myelosuppression	None	0.5–10
Mucositis	None	0.5–10

In the patient with meningeal lymphoma, fever to 101°F occurred after 5 days and was unassociated with headache or symptoms other than malaise. In this patient fever resolved within 2–3 days after discontinuation of the drug infusion, and on subsequent courses the febrile episodes progressively diminished and ultimately disappeared. Cultures of CSF and blood were taken at each febrile episode but did not disclose an infectious origin for the fever. No leukocytosis was seen in the peripheral blood although the CSF showed mild pleocytosis and slight increase in protein.

The first patient treated in this study received MTX (with methylparaben preservative) at a dose rate of 6 mg/day. This patient suddenly developed a transverse myelitis after 6 weeks of therapy. The patient reported intermittent leg weakness without objective signs for 2 weeks prior to onset of paralysis. CSF levels had been maintained between 12 and 30 μM in the 6 weeks prior to the onset of paralysis. All further patients received preservative-free MTX, and no further cases of leg weakness or transverse myelitis occurred.

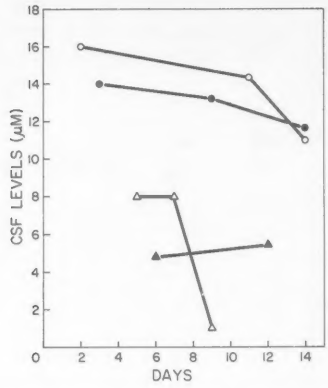

FIGURE 8.—Steady-state CSF levels of MTX in 4 patients during 1 infusion cycle of 7–14 days. ○ = patient 2, ▲ = patient 5, ● = patient 6, △ = patient 7.

After 3 months of therapy, two of the patients with astrocytomas displayed a diffuse hypodensity in the white matter on CT scan which was not associated with neurologic deficit. In one of the two patients, the hypodensity resolved while treatment continued.

As would be expected from the undetectable (< 0.01 μM) serum levels of MTX, no patients developed the side effects (stomatitis and myelosuppression) usually associated with systemic MTX toxicity.

Tumor Response

The patient with meningeal lymphoma had a complete response with total clearing of tumor cells from CSF and complete reversal of neurologic deficit (which had been manifest as a right leg paralysis). This patient has continued in a complete CSF remission for the 10 months since the start of treatment. This patient had retroperitoneal lymph node recurrence after 7 months, which was successfully treated with the addition of full-dose systemic chemotherapy while intraventricular therapy was continued.

Three of the five patients with astrocytomas had a 25% decrease in tumor size as determined by the product of the larger perpendicular diameters of enhancing area on CT scan while steroids were held

constant. These probable responses lasted 2, 3, and 6 months. Four of the five patients with astrocytomas subsequently received conventional cranial irradiation initiated 2–3 weeks after MTX had been discontinued. Three of the four patients tolerated radiotherapy well but subsequently died of tumor progression at 6, 6, and 14 months after the start of intrathecal therapy. The fourth patient did well for 2 weeks after radiotherapy and then rapidly deteriorated with increased intracranial pressure despite use of high-dose corticosteroids. In one patient with a grade IV astrocytoma (a "butterfly" glioma), the tumor calcified during MTX therapy alone.

DISCUSSION

The drug delivery system used in this study was found to be reliable and free of complications. There appear to be a number of important advantages to this system, which may make it applicable to a variety of therapeutic endeavors. Relatively constant drug levels can be maintained for extended, defined periods without the rapid changes in CSF drug concentrations that occur with repeated bolus injections. The natural composition of CSF is relatively unperturbed since the drug dose administered over 24 hours is infused in a volume of Elliott's B solution equivalent to only 1% of total daily CSF production. Sampling for ventricular drug level is readily accomplished through the reservoir. The administered solution goes through a 0.2-μ bacterologic filter before exiting the pump and entering the CSF, thus decreasing the potential for infections arising from the infusate. Of great importance from the patient's viewpoint is that the treatment is given on an outpatient basis without any of the restrictions on activity that are imposed by external hardware.

With this system, constant CSF levels of MTX were maintained that were at least 200- to 3000-fold higher than concurrent plasma levels. CSF levels of MTX were held in the 1–10-μM range without systemic toxicity. The lack of significant systemic toxicity such as myelosuppression and mucositis should make it possible to administer full doses of systemic chemotherapy in instances where systemic tumor elsewhere in the body requires control. The CSF levels of MTX exceed the estimated minimal cytocidal extracellular MTX concentration of 0.5 μM for leukemic cells (14). Therefore, it is possible with this system to maintain a cytotoxic level of MTX in CSF for periods far surpassing the cycle time of leukemia cells, which has been estimated to be about 3 days (15). Similar attempts by Bleyer et al (16) to maintain cytotoxic levels of MTX using a concentration × time (C × T) regimen of 1 mg twice daily demonstrated equal efficacy with much less neurotoxicity as compared to the conventional bolus injection of 12 mg/m² of MTX twice weekly. Bleyer suggested that the C × T approach is worthy of study in CNS neoplasms other than meningeal leukemia. We believe that the drug delivery system described herein is ideal for pursuing such studies.

Levels of MTX in CSF depend not only on the infusion dose rates used but also on the clearance of MTX from the CSF. The latter is dependent in part on CSF bulk flow (17), which may fluctuate with time and be variable from patient to patient. Variable CSF bulk flow could account for the minor variations of MTX levels seen in each patient and from patient to patient for a given dose rate of MTX. Disruption of the blood-brain barrier by the multiple brain metastasis in patient 3 may have allowed more efficient efflux of MTX from the CSF and resulted in lower MTX levels (fig 6). Other factors influencing the variability of MTX levels are the tolerances in formulation of the drug solution placed into the pump and the minor fluctuations in pump flow rates which occur with body temperature changes.

The most frequent toxic effects with the continuous intraventricular infusions of MTX were fever (seen in four of the seven patients) and meningism (seen in three). These side effects of intraventricular therapy have been described in numerous reports with an incidence ranging from 5% to 50% (18). The pathophysiology of this syndrome is unclear. In our study the incidence of fever had no clear dose relationship, and fevers occurred at dose rates as low as 0.5 mg/day.

The most severe toxic reaction was transverse myelitis occurring in our first patient. The patient received MTX with preservative (methylparaben). Whether the paraplegia was secondary to the preservative as proposed by Saiki et al (19) or was due to the maintained high concentrations of MTX in the CSF space as suggested by Bleyer et al (20) remains unclear. However, this complication did not occur in six subsequent patients in whom MTX levels were maintained, albeit at lower levels, using preservative-free drug.

Another side effect related to the use of continuous-infusion MTX was the finding in two patients of a CT scan abnormality noted as diffuse hypodensity of white matter regions of the brain. This radiologic abnormality could represent preclinical leukoencephalopathy as suggested by Peylan-Ramu et al (21). There was no concomitant deterioration of neurologic function with development of the CT scan abnormality in these two patients. This complication of intrathecal MTX therapy has been previously described in patients who received prior or

concomitant radiation therapy to the craniospinal axis (22–25). In the cases of leukoencephalopathy reported by Shapiro et al (25), the authors concluded that the explanation for the unique tomographic pattern of the lesions confirmed at autopsy was accumulation of toxic concentrations of drug in the periventricular tissues resulting from ventricular outflow obstruction. However, all patients in our current study had initial normal bulk flow of CSF as determined by pretreatment radionuclide cisternography.

The sustained response of the patient with meningeal lymphoma is illustrative of the advantages of this drug delivery system. The patient was able to receive chemotherapy as an outpatient with one visit per month to the clinic for pump refill. This was important due to the great distance of her home from the clinic. Also, as is true in most patients with meningeal lymphomatosis or carcinomatosis, there was ultimately a need for concomitant therapy to control the tumor elsewhere in the body. The use of MTX by this system is nonmyelosuppressive and therefore allows administration of full-dose systemic chemotherapy without added toxicity. Although the optimum treatment schedule is not yet clear for meningeal neoplasia, courses of continuous intraventricular infusion with intervening break periods were very well-tolerated and efficacious in our patient.

The C × T method of Bleyer et al (16) utilizing frequent multiple-drug injections into the ventricular reservoir may approximate a continuous infusion and may therefore be nearly as effective as our system for drug-sensitive meningeal neoplasms (lymphomas and leukemias). However, the implanted system should offer the potential for improved therapeutic efficacy in tumors having more intrinsic drug resistance by allowing maximal sustained drug exposure. In the future, a comparative trial of the multiple-dosing approach versus continuous intraventricular infusion will be warranted.

Although it is obvious that producing a high CSF drug concentration should be effective against meningeal disease, the bearing of CSF drug levels on the treatment of intraparenchymal brain tumors is controversial and not as straightforward. The production of a high CSF drug level has the potential to drive drug into brain and tumor parenchyma. Computer modeling studies and preclinical studies with rhesus monkeys indicate that maintaining an intraventricular level of MTX for 48–72 hours can lead to a drug concentration in the brain at 1 cm from the surface that is about one tenth of the CSF concentration (26–28).

However, other factors in addition to distance from the CSF surface could lead to inadequate drug exposure at the tumor. In particular, the one limiting factor to achieving effective drug levels within the tumor may be the loss of drug across the altered and more permeable peripheral capillaries of the tumor and surrounding brain tissue, which would prevent drug from reaching more centrally located tumor cells. Hence, inadequate drug exposure as well as intrinsic resistance could explain why the three responses in our patients with gliomas were minor and of short duration although MTX levels were maintained at presumably cytotoxic levels for prolonged periods of time. Hence, MTX does not appear to be highly efficacious against gliomas in the dose schedule and route employed in this study.

This drug delivery system is compatible for use with other agents of potential efficacy against CNS neoplasia. Clinical studies are under way with Ara-C, and preclinical studies in primates are planned for a variety of antineoplastic drugs. This system allows the precise maintenance of a given drug level and thus should allow extrapolation from preclinical studies into clinical protocols on a CSF drug concentration basis.

This system should have considerable applicability to serious nonneoplastic diseases. The use of constant intraventricular infusion of gentamicin in patients with chronic gram-negative meningitis or of amphotericin B in patients with fungal meningitis represents several such applications (29,30). Infusions of appropriate analgesics such as morphine or endorphins may be beneficial in cases of intractable, debilitating pain (31). It also has been noted recently that a variety of hormones may have central effects when introduced into CSF in small doses (32,33). Hence, this system should facilitate preclinical and clinical investigations in neoplastic and nonneoplastic states. We believe the current study demonstrates that the system is reliable, safe, and convenient and its use will allow examination of the therapeutic effects of a number of drugs and schedules in a variety of disease states.

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Diffuse Histiocytic Lymphoma After Histologic Conversion: A Poor Prognostic Variant[1,2]

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SUMMARY

Diffuse histiocytic lymphoma (DHL) developed in 14 patients with a different non-Hodgkin's lymphoma and one with chronic lymphocytic leukemia. The median time from the diagnosis of the first malignancy to the appearance of DHL was 47 months (range, 8–112). The most common presentation of histologic conversion was new or recurrent lymphadenopathy. Hypercalcemia was associated with the onset of the DHL in four of 15 patients. Eight patients with DHL after histologic conversion were treated with doxorubicin, vincristine, and prednisone with or without cyclophosphamide, but none achieved complete remission. Two other patients did achieve a complete response with therapy (one with radiotherapy and one with cyclophosphamide, vincristine, procarbazine, and prednisone), but they had brief remissions, and no patient in this study achieved long-term, disease-free survival. This was reflected in the short (12-month) median survival after histologic transformation to DHL. It appears that patients with DHL after histologic conversion have a worse prognosis than those with de novo DHL.

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Diffuse histiocytic lymphoma (DHL) can appear de novo or can arise in patients with another lymphoproliferative disorder (ie, histologic conversion), usually a different non-Hodgkin's lymphoma or chronic lymphocytic leukemia. Previous reports of patients with DHL after histologic conversion from another lymphoma have described short survival, but few patients have been treated with the aggressive chemotherapy regimens that have been shown to be potentially curative in DHL. However, Jones et al (1) at the National Cancer Institute (Bethesda, MD) reported three long-term survivors among 15 patients with DHL that developed during the course of another lymphoma, suggesting that aggressive treatment was beneficial (1). Recent studies have demonstrated that different histologic subgroups of DHL appear to have different prognoses (2,3). If patients with DHL after histologic conversion have a different prognosis than those with de novo DHL, it might be related to a difference in the proportion of the various histologic subtypes. In this report we describe 15 patients we have recently studied with DHL occurring during the course of another lymphoproliferative disorder.

PATIENTS AND METHODS

Fifteen patients diagnosed as having DHL at the University of Iowa Hospitals between January 1977 and December 1979 had another pre-existing lymphoid malignancy. During this time, 146 new cases of non-Hodgkin's lymphoma were seen at our institution. The diagnosis of DHL and of the other non-Hodgkin's lymphomas were confirmed by pathologic review using the criteria of Rappaport (4). There were no instances of mixed cell populations

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(ie, the original lymphoma and DHL both present in the same lesion) in this series. Our criteria for assigning histologic subtypes of DHL have been described previously (3). Briefly, lymphomas with > 75% large cleaved cells were called large cleaved, lymphomas with 25%–75% large cleaved cells and 25%–75% large noncleaved cells were called large cleaved and noncleaved, and lymphomas with > 75% large noncleaved cells were called large noncleaved. Lymphomas with plasmacytoid features (abundant amphophilic cytoplasm) were called immunoblastic sarcoma and other DHLs were unclassified. Surface marker studies and chromosome analyses were not performed on these lymphomas.

Stages were assigned by the application of the Ann Arbor Staging System at the time of the diagnosis of DHL (5). Stage assignment for the DHL at the time of histologic conversion was based on sites of proven or suspected involvement by DHL. Four patients each had one biopsy-proven site of persistent involvement by the previous lymphoma (three lymph node and one marrow) at the time of diagnosis of DHL, but those sites were not considered in assigning the stage of the DHL. Staging procedures after biopsy proof of histologic conversion were not uniform. However, 11 of the 15 patients had biopsy proof of extranodal (ie, stage IV) involvement. It is possible that the three stage II patients were understaged because further evaluation was not elected before beginning therapy. Complete remission (CR) was defined as the absence of clinically demonstrable disease after the completion of therapy and restaging. Persistent disease at sites of biopsy-proven involvement by the previous lymphoma in four patients never constituted the reason for treatment failure after treatment for DHL. Subsequent biopsies in several patients documented persisting DHL, never the original lymphoma, as the cause for treatment failure. The duration of survival was the time from diagnosis of DHL to death.

Three patients were treated every 3–4 weeks with doxorubicin (80 mg/m² iv), vincristine (1.4 mg/m² iv; maximum dose, 2.0 mg), and prednisone (100 mg/day orally for 5 days) (HOP). Five patients were treated every 3–4 weeks with cyclophosphamide (750 mg/m² iv), doxorubicin (50 mg/m² iv), vincristine (1.4 mg/m² iv; maximum dose, 2.0 mg), and prednisone (100 mg/day orally for 5 days) (CHOP). The planned program was six cycles, followed by restaging to determine treatment duration.

Survival of groups was calculated using the life-table method. Differences between life-tables were tested using log-rank analysis. Differences between groups were evaluated using the Fisher's exact test.

RESULTS

The clinical findings in the 15 patients are presented in table 1. The patients ranged in age from 48–73 years; there were eight males and seven females. The pre-existing non-Hodgkin's lymphoma was nodular poorly differentiated lymphocytic in eight patients, nodular mixed lymphocytic-histiocytic in four, nodular histiocytic in one, and diffuse well-differentiated lymphocytic in one. One patient had chronic lymphocytic leukemia. The interval from the diagnosis of the preceding non-Hodgkin's lymphoma or chronic lymphocytic leukemia to the appearance of the DHL ranged from 8 to 112 months (median, 47). All patients had received therapy for the preceding lymphoproliferative disorder; it consisted of combined chemotherapy and radiotherapy in eight patients, chemotherapy alone in five, and radiotherapy alone in two. Twelve patients were not receiving treatment at the time of histologic conversion and had been untreated for at least several months. Patient 5 was receiving oral cyclophosphamide at the time of appearance of DHL. Patients 1 and 8 had oral alkylating agents discontinued at 2 months and 1 month, respectively, before the histologic diagnosis of DHL.

The most common presentation of DHL was new and recurrent lymphadenopathy, seen in eight patients. Two patients had abdominal pain, two had fever, and three experienced weight loss. Intra-abdominal or retroperitoneal DHL was present in ten patients, 11 patients had extranodal disease, and 12 were stage III or IV. Four of the 15 patients developed symptomatic hypercalcemia coincident with the development of DHL.

Histologic subclassification revealed the predominant cell type to be large noncleaved in five patients, large cleaved in one, and large cleaved and noncleaved in nine. The survival of patients with predominantly large noncleaved cells was slightly better but not significantly different from the survival of those with mixed large cleaved and large noncleaved cells.

Fourteen patients received therapy for DHL and one died before treatment was started (table 1). Eight patients were treated with HOP and CHOP and none of these patients achieved CR. Only two brief CRs were achieved—one with radiation therapy (lasting 1 month) and one with cyclophosphamide, vincristine, procarbazine, and prednisone (lasting 4 months). No patient achieved long-term, disease-free survival. The median survival for the entire group is predicted by life-table analysis to be 12 months. Figure 1 shows the survival of the 15 patients in this series compared with that of 30 pa-

tients with de novo DHL we have recently treated with CHOP (6).

The toxic effects of therapy in this group of patients were similar to what we have observed previously (6). The most frequent toxic effect requiring dose modification was peripheral neuropathy resulting in reduction of the vincristine dose in two of eight patients treated with CHOP/HOP and in two other patients. There were no episodes of treatment-related fatal infection or hemorrhage. In the eight patients treated with CHOP/HOP, the percents of optimal dose actually administered were: 96%, cyclophosphamide; 94%, doxorubicin; 91%, vincristine; and 100%, prednisone. In 39 total courses of CHOP/HOP, the dose of the myelotoxic agents was reduced because of low blood cell counts seven times in six patients, and the dose of vincristine was reduced a total of 13 times in six patients. These findings are not different from our experience in patients treated for de novo DHL.

DISCUSSION

The occurrence of aggressive, large cell lymphomas in patients with pre-existing but less aggressive non-Hodgkin's lymphomas and chronic lymphocytic leukemia has been recognized for many years (7,8). Most of these tumors appear to be DHL. The frequency rate of the phenomenon has been found to be 23% in 80 patients with nodular poorly differentiated lymphocytic and nodular mixed lymphocytic-histiocytic lymphoma (9), 22% in 55 patients with follicular lymphoma (10), 27% in 30 patients with low-grade, non-Hodgkin's lymphoma (11), and at least 3% in 151 patients with chronic lymphocytic leukemia (12). It is not clear whether these tumors represent a transformation of the preexisting neoplasm analogous to blast cell crisis in chronic granulocytic leukemia or are a second, independent neoplasm, and it is not clear to what extent preceding cytotoxic therapy plays a part in their development. Studies of surface markers have been interpreted as being consistent with (13) and inconsistent with (14) derivation from the original malignant clone. Perhaps both possibilities are operative in various cases.

Previous reports of aggressive large cell lymphomas developing in patients with other non-Hodgkin's lymphomas have identified new lymphadenopathy (11), change in the hardness of lymph nodes (10), change in response to therapy (10,11), and the development of a leukemic phase (10) as the presenting manifestations. In nine cases of DHL appearing in patients with chronic lymphocytic leukemia, the onset of the lymphoma was heralded by abdominal symptoms, increasing lymphadenopathy, hepatomegaly, and splenomegaly (12). The patients in this study do not differ from those previously reported except that none presented with a leukemic picture. New or enlarging lymphadenopathy was the most common presentation of DHL and the most common reason for rebiopsy. The frequency of intra-abdominal or retroperitoneal disease was high. There was a relatively higher incidence of hypercalcemia than has been generally observed for non-Hodgkin's lymphomas. However, hypercalcemia in non-Hodgkin's lymphoma appears to be most frequently seen in the diffuse histiocytic category, particularly when there is bone involvement (15).

It has recently become apparent that histologic subgroups of DHL might have different prognoses (2,3). We have described a system of histologic subtyping that, when applied to de novo DHL, determined that 23% of the lymphomas had large cleaved cells, 23% had large noncleaved cells, 37% had mixed large cleaved and noncleaved cells, 7% were immunoblastic sarcoma, and 10% were unclassified (3). Although the frequency of occurrence of large cleaved cells was slightly less in DHL after histologic conversion (7%) than in de novo DHL, the frequencies of the other subtypes were similar. Thus, the poor prognosis found in DHL after histologic conversion from another lymphoma does not appear to be explained by differences in histologic subtypes.

Although the histologic transformation of less aggressive non-Hodgkin's lymphomas and chronic lymphocytic leukemia into DHL has generally been found by previous authors to be associated with a poor prognosis, very few of the reported patients have been treated with the intensive chemotherapy regimens that have recently been shown to produce long-term, disease-free survival in a significant proportion of the patients with DHL. Jones et al (1) reported 15 patients who were treated aggressively (exact regimens not specified), with a median survival for the entire group of 9.5 months (1). Five patients achieved CR and 3 were surviving at > 47 months. Eight of the 15 patients in the present series received intensive therapy with CHOP or HOP. In addition, we treated two other patients with CHOP who presented in the same time period with a good prognosis non-Hodgkin's lymphoma and DHL discovered concurrently and who might have been examples of unrecognized histologic conversion. Unfortunately none of these patients achieved CR. This is in contrast to 15 CRs in 30 patients with de novo DHL we have treated with CHOP (6). Two patients with DHL after histologic conversion who

TABLE 1.—Clinical, histologic, and therapeutic findings

Patient No.	Age	Sex	Sub-type*	Stage	Previous treatment†	Time to appearance of DHL (mos)	Presentation‡	Sites of disease§	Stage	Histologic subtypes¶	Study treat-ment†	Re-sponse**	Duration of CR (mos)	Duration of survival (mos)††	Comments
1	57	F	NPDL	IV	COP, XRT	93	↑ nodes	N	II	LCNC	HOP XRT COMA	NR NR PR	—	18+	
2	50	M	NPDL	IV	Chlor, COP	84	Fever, wt loss	M, L	IV	LNC	HOP BVP	PR PR	—	5+	
3	48	M	NPDL	III	COP, cyclo	72	Weakness	M	IV	LCNC	CHOP MOPP COMA	PR PR NR	—	9+	Hypercalcemia
4	67	M	NPDL	I	XRT	72	↑ nodes	N, M	IV	LNC	CHOP CDDP CCNU XRT	PR NR NR NR	—	12	Developed CNS DHL
5	54	M	NPDL	III	Cyclo	71	Dyspnea, dysphagia, mediastinal mass	N, S, P	IV	LNC	BVP Doxo XRT CDDP	NR PR NR NR	—	8	Hypercalcemia
6	70	M	NPDL	IV	COP, cyclo, XRT	47	Wt loss, night sweats, abd pain	RP	IV	LCNC	None	—	—	<1	Hypercalcemia
7	61	M	NPDL	IV	MOPP, cyclo, XRT	36	Fever, leg edema, abd mass	N, S	II	LCNC	BCOPP XRT	PR NR	—	4	
8	55	M	NPDL	III	TBI + COP, chlor	18	↑ nodes, back pain, cord compression	N, M	IV	LCNC	XRT	PR	—	1	
9	69	F	NM	III	CHOP, C-MOPP	42	Scalp nodule	D, O, CNS	IV	LC	XRT CHOP COPP	CR PR NR	1 —	7+	Chylous pleural effusions
10	70	F	NM	IV	TBI, COP	27	↑ nodes, wt loss	N, M	IV	LCNC	HOP VBP	PR NR	—	17	
11	56	F	NM	III	TBI + COP, doxo, ICRF-159, CDDP	24	↑ nodes, chest wall mass	H, N, sc mass	IV	LNC	CDDP Vinb	PR NR	—	7	Chylous pleural effusions
12	58	M	NM	IV	C-MOPP	8	Abd pain, abd mass	RP	IV	LCNC	CHOP Bleo XRT	NR NR PR	—	5	
13	63	F	NH	I	XRT, COP	30	↑ nodes	N	III	LNC	CHOP XRT Vinb	PR PR PR	—	20+	
14	69	F	DWDL	I	XRT	112	↑ nodes	N	II	LCNC	C-MOPP Adria	CR NR	4	12	Hypercalcemia
15	73	F	CLL	II	COP, TBI	102	↑ nodes, breast mass	N B	IV	LCNC	ABP CDDP	PR NR	—	13	

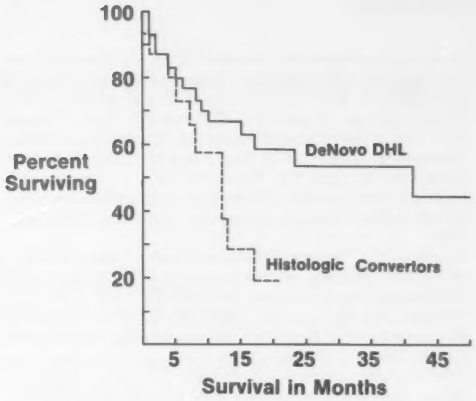

FIGURE 1.—Survival of 15 patients with DHL after histologic conversion is depicted using the life-table method and compared to that of 30 patients with advanced de novo DHL.

did achieve a CR after therapy (one with radiotherapy and one with C-MOPP) had brief remissions lasting only 4 months and 1 month. This is unlike the frequent durable CRs we have seen in de novo DHL (6). The paucity of CRs is reflected in the short survival of patients with DHL after histologic conversion (median, 12 months) (fig 1).

The obvious explanation for the poorer outlook observed in patients with DHL after histologic conversion than in those with de novo DHL, despite comparably intensive therapy, is the effect of preceding cytotoxic therapy. The DHL that develops in patients with Hodgkin's disease after treatment with radiotherapy and chemotherapy and the acute leukemias that develop in patients after radiotherapy and/or alkylating-agent chemotherapy also respond poorly to treatment (16,17). However, not all patients with DHL following another lymphoproliferative disorder have received previous treatment, and it may be that DHL, after histologic conversion, is a more aggressive manifestation of the original neoplasm (ie, malignant transformation) rather than an entirely new disease.

In summary, despite aggressive therapy, patients with DHL occurring during the course of another lymphoproliferative disorder appear to have a lower incidence of CR, a shorter median survival, and a paucity of long-term, disease-free survival, compared to those with de novo DHL. The difference in outcome does not appear to be explained by differences in histologic subtypes.

*NPDL = nodular poorly differentiated lymphocytic; NM = nodular mixed lymphocytic-histiocytic; NH = nodular histiocytic; DWDL = diffuse well-differentiated lymphocytic; CLL = chronic lymphocytic leukemia.
†COP = cyclophosphamide, vincristine, and prednisone; XRT = radiation therapy; chlor = chlorambucil; cyclo = cyclophosphamide; MOPP = mechlorethamine, vincristine, procarbazine, and prednisone; TBI = total-body irradiation; C-MOPP = cyclophosphamide, vincristine, procarbazine, and prednisone; doxo = doxorubicin; CDDP = cisplatin; COMA = cyclophosphamide, vincristine, methotrexate, and cytarabine; BVP = bleomycin, vinblastine, and cisplatin; BCOPP = bleomycin, cyclophosphamide, vincristine, procarbazine, and prednisone; vinb = vinblastine; bleo = bleomycin; ABP = doxorubicin, bleomycin, and prednisone.
‡↑ = increased. No. and size; abd = abdominal.
§N = lymph nodes; M = bone marrow; L = lung; S = spleen; P = pleura; RP = retroperitoneum; D = skin; O = bone; H = liver; B = breast.
¶LCNC = large cleaved and noncleaved; LNC = large noncleaved; LC = large cleaved.
**NR = no response.
††Survival time from the diagnosis of histologic conversion to DHL.

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Prospective Study of the Pulmonary Toxicity of Continuously Infused Bleomycin[1]

Kevin R. Cooper[2,*] and Waun K. Hong[3,4]

SUMMARY

Bleomycin is an effective antitumor drug but must be used cautiously because of its pulmonary toxicity. We performed a prospective study of the effects on pulmonary function when bleomycin was given as a continuous iv infusion over a 7-day period. Bleomycin was administered at a dose of 15 units/m² as an iv push followed by 15 units/m²/day as a continuous iv drip. Thirteen previously untreated patients completed the study after receiving a mean total dose of 228 units. Pulmonary function tests were performed before bleomycin treatment and periodically during the subsequent 6 months. There was a small mean *increase* in both vital and diffusing capacities after 6 months, which was not statistically significant. No patient had a serious loss in pulmonary function. We conclude that the risk of bleomycin toxicity is low when the drug is given at this dose by continuous infusion. The risk may be less than the risk of toxicity from bolus injection in this dose range.

[Cancer Treat Rep 65:419–425, 1981]

Bleomycin is being used with increasing frequency for the treatment of squamous cell carcinoma, testicular cancer, and lymphomas (1). It has most often been administered im or as a rapid iv infusion at intervals of 1–7 days, but recently, continuous iv infusion of bleomycin over several days is undergoing clinical trials. There is considerable evidence that continuous infusion may be the most effective method for giving bleomycin alone (2–4) or in combination chemotherapy (5,6) because it is cell-cycle specific and has a short half-life (7,8).

The principal toxic effects of bleomycin are mucocutaneous inflammation and interstitial pneumonia. The latter may lead to pulmonary fibrosis and restrictive lung disease. Pulmonary side effects were recognized very early in the development of bleomycin and these effects often limit its use. The risk of pulmonary toxicity is higher for older patients and for those receiving doses > 450 units (9).

Most experience with bleomycin pulmonary toxic effects in man is based on intermittent administration of the drug over several months. A recent report by Comis et al (10) emphasizes the hazard of bleomycin when it is given by weekly bolus injection. Their patients had a significant loss of pulmonary diffusing capacity after only two bolus injections of bleomycin. In addition, most patients who have developed bleomycin pulmonary toxic effects have also received other chemotherapy or radiation therapy, which increases the pneumotoxic effects of bleomycin (11,12). We conducted a prospective 6-month study of effects of continuous infusion with bleomycin on pulmonary function tests (PFTs), chest radiographs, and arterial blood gases in a group of previously untreated patients to determine the pulmonary toxic effects of this particular drug regimen.

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TABLE 1.—Patient characteristics*

Patient No.	Age (yrs)	Tumor type and stage	Pre-existing pulmonary disease	Chemotherapy regimen	Total dose of bleomycin
1	64	PDSC, pharynx, $T_2N_3M_0$	Moderate COPD	Standard	224
2	50	WDSC, tongue, $T_2N_2M_0$	Documented history of multiple pulmonary emboli	Standard	208
3	57	PDSC, oropharynx, $T_3N_0M_0$	Moderate COPD	Standard	165
4	46	WDSC, pharynx, $T_3N_0M_0$	None	Standard	240
5	57	WDSC, tongue, $T_3N_0M_0$	Mild COPD	Standard	232
6	50	WDSC, tongue, $T_3N_2M_0$	None	Standard	256
7	53	Choriocarcinoma of retroperitoneum	Multiple pulmonary metastases	Standard + CVD	215
8	68	PDSC, pharynx, $T_4N_3M_x$	Recurrent aspiration pneumonia	Standard	208
9	58	PDSC, tongue, $T_3N_2M_x$	None	Standard	240
10	24	Embryonal cell cancer, testicle	None	Standard + CVD	289
11	70	WDSC, pharynx, $T_4N_0M_0$	Mild COPD	Standard	216
12	58	WDSC, oropharynx, $T_2N_2M_0$	Mild COPD	Bleomycin only	228
13	52	PDSC, esophagus and WDSC, oropharynx, $T_4N_2M_0$	Mild COPD	Standard	216
14	51	WDSC, oropharynx, $T_4N_1M_0$	Mild COPD	Standard	240
15	70	PDSC, pharynx, $T_3N_1M_0$	Moderate COPD	Standard	224

*WDSC = well-differentiated squamous carcinoma; PDSC = poorly differentiated squamous carcinoma; COPD = chronic obstructive pulmonary disease; standard = bleomycin and cisplatin; CVD = cyclophosphamide, vinblastine, and dactinomycin.

MATERIALS AND METHODS

Fifteen male patients were entered in the study. Descriptive data are presented in table 1. Thirteen patients had squamous carcinoma of the tongue or pharynx and were treated with a regimen of bleomycin and cisplatin. The dose of bleomycin was 15 units/m² as a slow iv push followed by 15 units/m²/day for 7 days as a continuous iv infusion. Two patients with germ cell tumors were also treated with this regimen and, in addition, received cyclophosphamide, vinblastine, and dactinomycin. No patient had previously received therapeutic radiation, chemotherapy, or any agent known to be toxic to the lung. The mean dose of bleomycin for all 15 patients was 227 units.

To discover any pre-existing lung disease, each patient was interviewed and examined by a pulmonary physician before bleomycin was given. During the course of the study, patients were regularly evaluated for signs and symptoms of bleomycin pulmonary toxic effects. PFTs were carried out before the treatment and 1, 3, and 6 months after treatment. PFTs consisted of spirometry, lung volume measurement by the helium dilution technique, and single-breath carbon monoxide diffusing capacity (Maxi-system, Warren E. Collins, Braintree, MA). Arterial blood gases, hemoglobin levels, and routine posteroanterior and lateral chest radiographs were obtained before and 6 months after chemotherapy.

The results of the PFTs were analyzed statistically by a one-way analysis of variance test. Arterial blood gas values before and 6 months after bleomycin were evaluated by the Student's t test. Chest radiographs were carefully examined for interval changes by a pulmonary physician and were then reviewed in detail with a chest radiologist.

RESULTS

Thirteen patients completed the 6-month study. The mean total dose of bleomycin was 228 units. Two patients (Nos. 8 and 15) died before serial PFTs could be performed. Their deaths were due to aspiration pneumonia and extensive brain metastases, respectively. Neither of these patients had clinical signs of bleomycin pulmonary toxic effects but no followup PFTs were performed.

Three of our patients required permanent tracheostomies as part of the surgical therapy of their upper airway tumors. All tracheostomies were done after the baseline PFTs had been performed. There was no evidence by clinical examination or flow volume loops that these patients had had upper airway obstruction.

Two of the 15 patients (13%) had a complete response, seven (47%) experienced a partial response, and six (40%) showed no response. Dermatitis attributed to bleomycin was seen in two patients (one

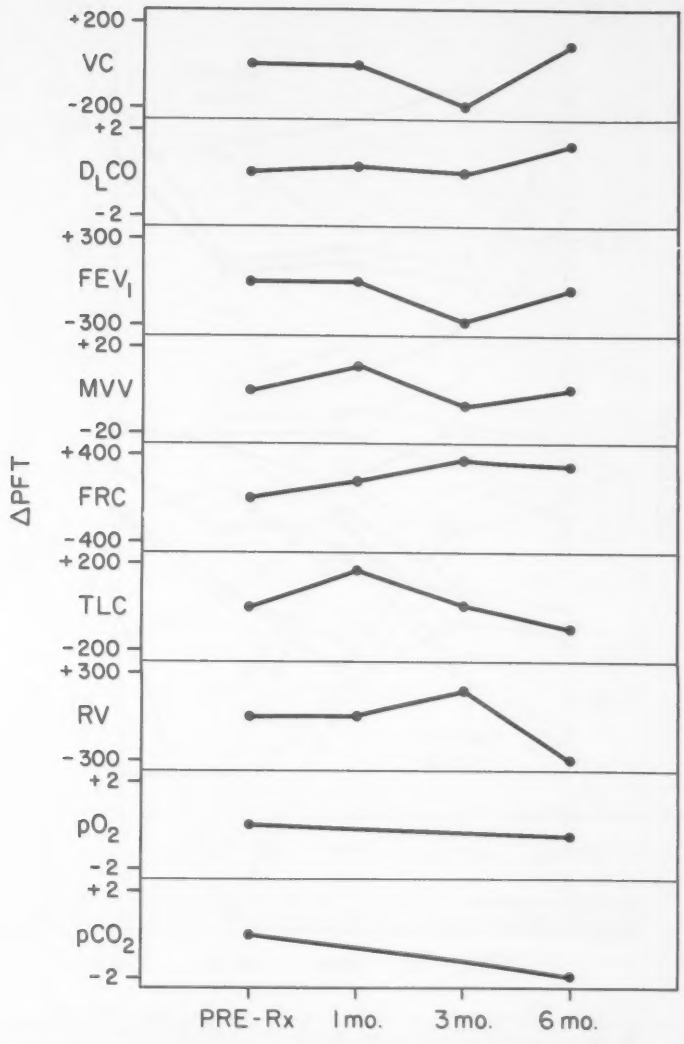

FIGURE 1.—Mean change in PFTs for the entire group. All units = standard units of measurement for PFT.

mild and one moderate) and mucositis was seen in nine patients (eight mild and one moderate).

PFT Results

The 13 surviving patients were analyzed as a group for changes in vital capacity (VC), forced expiratory volume in 1 second, maximal voluntary ventilation, functional residual capacity, total lung capacity, residual volume, pulmonary diffusing capacity (D_LCO), PaO_2, and $PaCO_2$. These tests showed no statistically significant alterations over time, compared to prebleomycin values (fig 1).

All individual values for VC and D_LCO are shown in figures 2 and 3, respectively. Four patients showed some decline in VC (fig 2). Patient 2 showed

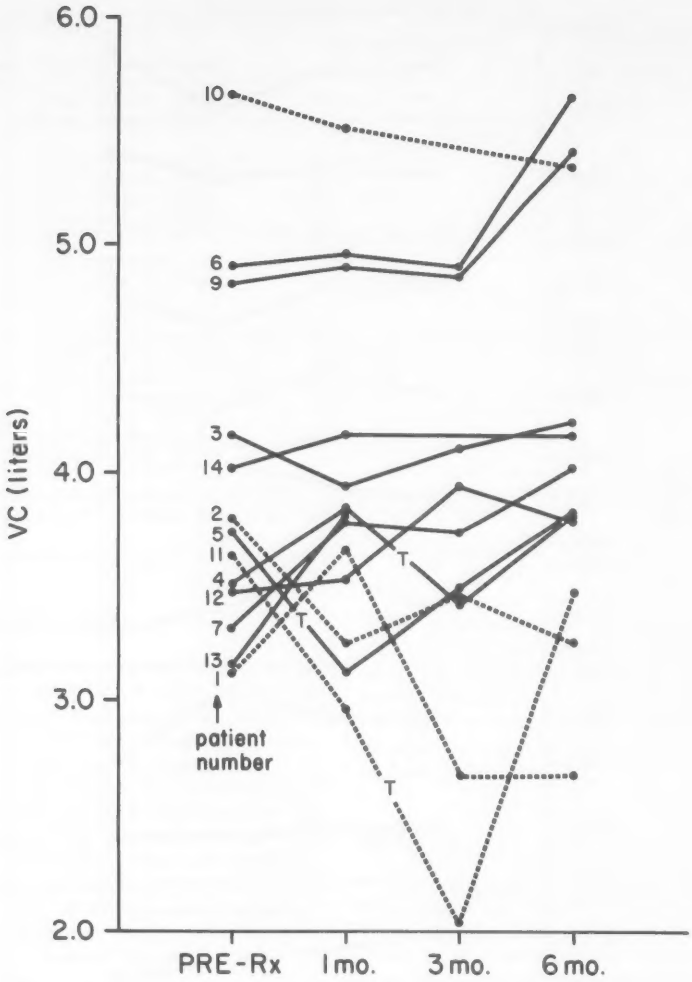

FIGURE 2.—Change in VC for each patient. T = tracheostomy performed; ●—● = VC improved after 6 mos; ●----● = VC decreased after 6 mos.

a loss of VC and an improvement of D_LCO. Patient 11 had large decreases in VC and D_LCO which were clearly related to an episode of aspiration pneumonia. His VC largely recovered by the end of the study, but his D_LCO remained low. Patients 1 and 10 showed modest decreases in VC and D_LCO which were clinically compatible with bleomycin toxic effects but both patients were asymptomatic.

Other Studies

There was no change in group values for PaO_2 or $PaCO_2$ over the 6-month period. No patient developed hypercapnia ($PaCO_2 > 45$ torr) or severe hypoxemia ($PaO_2 < 55$ torr). Hemoglobin levels were also unchanged for the group, and individual changes did not correlate with changes in D_LCO.

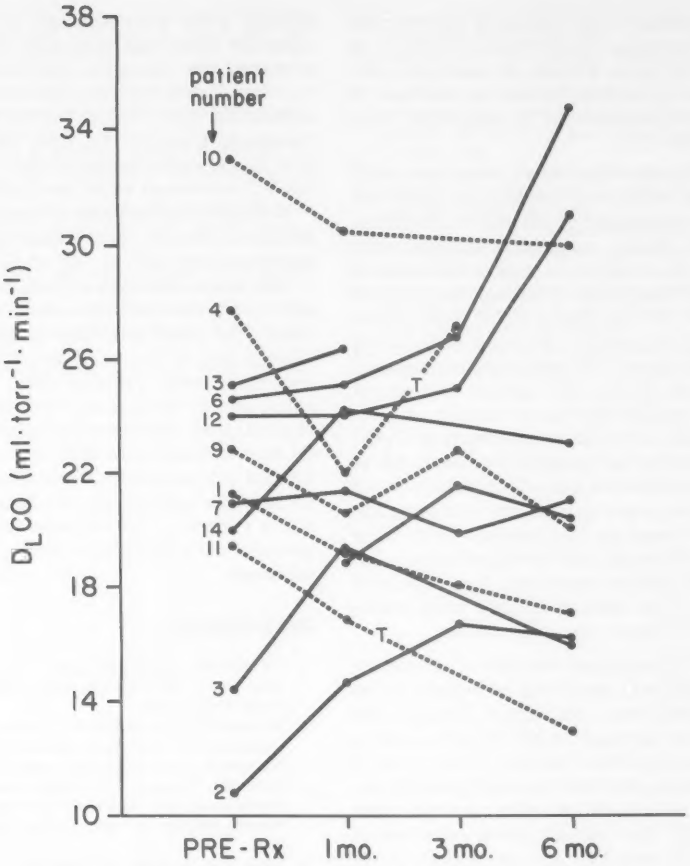

FIGURE 3.—Change in D_LCO for each patient. T = tracheostomy performed; ●—● = D_LCO improved after 6 mos; ●----● = D_LCO decreased after 6 mos.

Comparison of baseline and postbleomycin chest radiographs showed that three patients (Nos. 2, 7, and 13) had new interstitial infiltrates in a fine reticulonodular pattern. In each case, the infiltrate was slight and unilateral.

DISCUSSION

A great deal is known about the clinical (13–16), pathologic (17), and cytopathologic (18) aspects of bleomycin-induced pulmonary toxicity. However, it is not certain whether only the total dose of bleomycin or also the route and timing of administration affects the incidence of pulmonary toxicity. Interaction between single-course bleomycin and other types of cancer treatment may also be very important. It has been shown that the risk of bleomycin toxic effects is greater in patients who have received radiotherapy (11) or a prior course of bleomycin (12).

Other chemotherapy agents may also have cumulative or even synergistic toxic effects in man when they are given with or before bleomycin. Mice treated with combinations of bleomycin and cisplatin or bleomycin and vincristine develop greater pulmonary toxic effects than control animals treated with bleomycin alone (19). All but one of our patients received cisplatin which has not been asso-

ciated with pulmonary toxic effects of its own (20) but which could enhance bleomycin toxic effects in man as it does in mice. Patient 10 received substantial doses of cyclophosphamide in addition to bleomycin. Cyclophosphamide is associated with pulmonary fibrosis (21).

Comis et al (10) described nine young men with no prior therapy who were treated for germ cell tumors with a combination of bleomycin, cisplatin, and vinblastine. Thirty units of bleomycin were given at weekly intervals and a steady dose-related decrease in D_LCO was noted. Although the greatest decline was seen at total doses > 240 units, there was a statistically significant decrease in D_LCO at every cumulative dose > 60 units when compared with pretreatment D_LCO. Comparison of their results with ours implies that slow infusion of bleomycin is less likely to result in a decrease in D_LCO than is bolus injection at comparable doses. Alternatively, the simultaneous use of vinblastine could have enhanced the pulmonary toxicity of bleomycin. Comis et al also mention two patients with prior mediastinal radiotherapy who developed severe reductions of D_LCO after a mean total dose of only 60 units, supporting the contention that prior radiotherapy enhances bleomycin toxic effects.

Pascual et al (14) reported the loss of pulmonary function of 14 patients receiving bleomycin twice weekly (mean total dose, 262 units). This patient group had a mean decrease in VC of 433 ml and a mean decrease in D_LCO of 4 $ml \cdot torr^{-1} \cdot min^{-1}$. All of these patients, however, had received previous radiotherapy, and two thirds had also received other chemotherapy (22). Our patient group experienced mean increases in both VC (150 ml) and D_LCO (1 $ml \cdot torr^{-1} \cdot min^{-1}$) after 6 months. Thus, the loss of pulmonary function observed by Pascual et al was probably due to the toxicity of bleomycin plus other therapy, rather than to that of bleomycin alone.

We believe that the changes in VC and D_LCO over the followup period represent real changes for each individual rather than random variations, since both of these tests yield quite reproducible results. Moreover, there is no reason to think that patients undergoing treatment for cancer will maintain the same VC and D_LCO over a 6-month period even if the accuracy of the testing procedure is perfect, since some patients are responding to therapy, some are having ill effects from progressing malignancy, and some are developing intercurrent illness or complications of therapy.

Although we recognize that chest radiography is neither sensitive nor specific in detecting bleomycin toxicity, chest films taken before and at least 6 months after chemotherapy were reviewed. We carefully examined each pair of films, specifically searching for changes consistent with bleomycin toxicity. Three patients had radiographic changes compatible with, though certainly not diagnostic of, bleomycin toxic effects. Only one of these patients (No. 2) showed a decline of either VC or D_LCO, and his VC decreased while his D_LCO improved.

It would be premature to suggest that continuous infusion of bleomycin at a dose of 228 units is totally safe for every patient. All of our patients received < 400 units; although a high incidence of serious pulmonary toxic effects is not expected at this low dose, even small doses are capable of inducing lung disease due either to direct toxic effects or to a hypersensitivity reaction (23). However, our results support the findings of Sikic et al (3) and Baker et al (6) that continuous infusion of bleomycin may be less toxic than intermittent bolus injection. Since none of our patients suffered clinically serious bleomycin toxic effects, we conclude that this regimen has a very low risk of pulmonary toxic effects in patients with no prior radiation therapy or chemotherapy.

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Rubidazone in Adults With Previously Treated Acute Leukemia and Blast Cell Phase of Chronic Myelocytic Leukemia: A Southwest Oncology Group Study[1,2]

J. Bickers, R. Benjamin, H. Wilson, H. Eyre, J. Hewlett, and K. McCredie[3,*]

SUMMARY

Rubidazone, a new anthracycline antibiotic, is the benzoyl hydrazone derivative of daunorubicin. The Southwest Oncology Group carried out a phase II study of the drug in 126 patients with previously treated acute leukemia; 116 patients were evaluable. Good-risk patients were given doses of 450 mg/m², and poor-risk patients were given doses of 300 mg/m² approximately every 3 weeks. No complete response was observed in 25 patients with chronic myelocytic leukemia blast cell transformation. In the remaining patients the overall complete response rate was 22% and the rate of complete plus partial response was 29%. In good-risk patients these rates were 27% and 35%, respectively. Toxicity was similar to that observed with other anthracyclines except that acute febrile reactions were more pronounced. On the basis of the results, there are plans for a large-scale comparison study of rubidazone versus doxorubicin, each in combination with cytarabine, vincristine, and prednisone.

[Cancer Treat Rep 65:427–430, 1981]

Rubidazone is the benzoyl hydrazone derivative of daunorubicin. Daunorubicin and its metabolites are in vivo metabolites of rubidazone in man.

Jacquillat et al (1) achieved complete remission (CR) in 57% of 70 previously untreated adults with acute leukemia, using a 5-day course of rubidazone given every 3 weeks. Benjamin et al (2) studied 39 patients previously treated with various dose schedules, achieving an overall CR rate of 33%. In 19 patients receiving the dose that was judged to be optimum, 300–450 mg/m² every 21 days, the CR rate was 53%.

Based on the preceding experiences, the Southwest Oncology Group (SWOG) carried out a phase II study of rubidazone in adults with previously treated acute leukemia or chronic myelocytic leukemia blast cell transformation (CMLBT).

MATERIALS AND METHODS

Adult patients with previously treated acute leukemia in relapse or with CMLBT were eligible for the study. The sum of marrow blast cells plus promyelocytes was required to be ≥ 20%, with demonstrated evidence of progressive disease. Eligibility was limited to patients who had received < 200 mg/m² of doxorubicin or the estimated equivalent of daunorubicin.

Informed consent was obtained in writing from each patient according to policies set forth by individual institutions.

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[3]This study was conducted by the Southwest Oncology Group. The following members participated in the study: Louisiana State University Medical Center, New Orleans (*J. Bickers*); M. D. Anderson Hospital and Tumor Institute, Houston, TX (*R. Benjamin and K. McCredie*); Ohio State University Hospital, Columbus (*H. Wilson*); University of Utah Medical Center, Salt Lake City (*H. Eyre*); and Cleveland Clinic Foundation, OH (*J. Hewlett*).

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In good-risk patients the initial rubidazone dose was 450 mg/m². Patients > 50 years old who had active infections or hypocellular marrows (< 50% cellularity) were judged to be poor risks and started at a dose of 300 mg/m². The drug was prepared in accompanying buffer, added to 500 mg of 5% dextrose in distilled water, and given iv over a 1-hour period. Premedication with antihistamine and allopurinol was recommended.

Bone marrow examination was repeated on Day 12 and every 3–4 days thereafter until antileukemic effects could be judged with certainty. This normally permitted re-treatment between Days 16 and 25. A 20% dose increment was specified if the marrow remained hypercellular and frankly leukemic, and a 20% dose reduction was specified if sepsis followed the first dose. Subsequent remission induction doses were given at similar intervals, using the same criteria.

If there was no evidence of reduction in leukemic cellularity after two doses of rubidazone, the patient was judged to be a treatment failure and removed from the study. If leukemic cellularity was reduced after each of the first two doses, additional doses were given in accordance with the judgment of the investigator.

Patients achieving a CR, according to previously published criteria (3), were given a consolidation dose of 300 mg/m² and then 150–200 mg/m² every 3 weeks until the maximum total dose of anthracycline was received. Additional remission maintenance chemotherapy was given in accordance with the judgment of the investigator, based on the patient's prior chemotherapy.

Patients had daily cbcs, including platelet counts. Hepatic and renal function tests were performed weekly. Prior to administration of each dose of rubidazone, an electrocardiogram was performed and the total QRS voltage in the six limb leads was summed.

In patients with prior anthracycline therapy, the total rubidazone dose was not permitted to exceed 1500 mg/m². Patients with no prior anthracycline therapy were permitted a total dose of 3500 mg/m². Cessation of rubidazone therapy was specified if there was a 30% reduction in the summed QRS voltage or if congestive heart failure developed.

Midway through the study, the treatment protocol was modified for patients with CMLBT. Vincristine (2 mg iv) was given on Day 1 and every 7 days thereafter until there was remission, criteria for treatment failure, or neurotoxicity. In addition, 200 mg of prednisone/day was given orally for 14 days, and then 100 mg/day was given until response or failure was documented.

RESULTS

A total of 126 patients were entered in the study. Ten patients were judged inevaluable because of inadequate data, failure to follow protocol, or ineligibility. The remaining 116 patients were analyzed. The breakdown of these 116 patients by diagnosis and risk status is given in table 1.

Except for those with CMLBT, all patients had had extensive prior chemotherapy. Eighty-five of the 91 had had prior anthracycline therapy, chiefly in the form of doxorubicin. In 71 patients, there were adequate data to judge response to prior therapy; 56 had had prior CR, five had had partial response (PR), and ten had had no response.

The response rate to rubidazone therapy is shown in table 1.

The overall CR rate was 17%, and the rate of CR plus PR was 22%. No patient with CMLBT achieved CR. One PR lasting 9 weeks was observed. Median survival from start of rubidazone therapy in CMLBT patients was 7 weeks. Because the therapy for CMLBT was different from that for the other acute leukemias and because the response rates in acute myelocytic leukemia (AML), acute lymphocytic leukemia (ALL), and unclassified acute leukemias were significant, these groups will be analyzed separately.

The CR rate in AML, ALL, and unclassified acute leukemias was 22%, with an overall response

TABLE 1.—Patient characteristics and rubidazone response

Disease	No. of patients				
	Evaluable (total)	Good risk		Poor risk	
		Evaluable	Response	Evaluable	Response
AML	67	54	CR, 13 PR, 3	13	0
ALL	15	14	CR, 4 PR, 3	1	0
Unclassified acute leukemia	9	7	CR, 3	2	0
CMLBT	25	12	PR, 1	13	0

rate of 29% including PRs. If only good-risk patients are considered, the CR rate was 27% and the rate of CR plus PR was 35%. The difference in the CR rates between good- and poor-risk patients approached statistical significance ($P = 0.098$, two-sided test). If total response rate was tested, the rates for good-risk patients were significantly higher ($P = 0.05$, one-sided test). In patients who had previously achieved CR, the CR rate to rubidazone was 22% and the rate of CR plus PR was 25%. Patients who had previously achieved less than a CR had a response rate of 7% ($P = 0.17$, two-sided test).

Median survival from start of rubidazone therapy (excluding patients with CMLBT) was 12 weeks in good-risk patients, 5 weeks in the poor-risk group, and 12 weeks for the entire group. The difference in survival between the good- and poor-risk groups was statistically significant ($P = 0.01$).

The median time needed to achieve CR was 4 weeks, with 80% of those with CR achieving M1 marrows after a single treatment. The longest time to CR was 10 weeks. Median duration of CR was 18 weeks, with a range of 4–36 weeks.

Toxic effects are notably difficult to assess in adult acute leukemia, and the problem is amplified in patients in relapse after prior therapy. In this study there was a strong overall impression that toxicity was consistent with that observed for other cytotoxic agents active in acute leukemia.

Myelosuppression was the most common toxic effect reported. In good-risk patients, the median leukocyte nadir following the first dose was 600/mm^3, and in poor-risk patients, it was 1400/mm^3. These values were the same for all cell types of leukemia. Thrombocytopenia tended to parallel leukopenia in severity but was not objectively evaluable because of platelet transfusions. Although time of re-treatment was based primarily on leukemia status, this nevertheless provided the most accurate index of duration of myelosuppression; in good-risk patients the median time of the second dose was Day 24 and in poor-risk patients, it was Day 17. One fatality appeared to be clearly related to drug-induced myelosuppression, with death occurring 53 days after the first dose, without evidence of marrow recovery. Two deaths, on Days 21 and 36, were probably related in part to drug-induced myelosuppression; neither patient had marrow regeneration, but leukemic blast cells persisted in both. In 14% of the patients, acute reactions immediately following administration of rubidazone consisted of chills, fever, tachycardia, and varying degrees of generalized erythema. Typically, these began 1–2 hours after therapy and lasted 6–12 hours. One such patient developed seizures and cardiac arrest 6 hours after treatment and was believed to have had a drug-related death. In another patient, severe fever and tachycardia with skin rash, which lasted 48 hours, were considered life-threatening and the patient was not re-treated.

Nausea and vomiting were reported in 17% of the patients; all but one were in the good-risk group. These side effects were generally mild to moderate, with only one patient rated as having severe nausea and vomiting.

Mucositis was reported in only three patients. In one, there was severe hemorrhagic pharyngitis, and another had severe hemorrhagic colitis that was judged to be drug-related. The third patient had moderate stomatitis.

Cardiotoxic effects were reported in one patient but with little supportive evidence. It is clear, however, that the patient died of noncardiac complications of leukemia.

Severe angioneurotic edema with airway obstruction developed in one patient who ultimately died on Day 13. Although timing was consistent with rubidazone reaction, it is impossible to be certain in a setting which involved numerous drugs in a severely ill patient.

It is clear that phlebitis secondary to rubidazone was not uncommon, but it was reported to be severe in only one patient.

One patient developed severe hepatic dysfunction after the first dose of rubidazone, requiring removal from the study. No causative role could be assigned to the drug, however. All patients who survived longer than 2 weeks developed total alopecia.

DISCUSSION

This phase II study confirms significant activity of rubidazone in acute leukemia and provides substantial data regarding biologic effects of the schedule used. It demonstrates the feasibility of the dose of 450 mg/m^2 every 3 weeks and suggests that this is close to the optimum dose for that schedule. Any interpretation of the therapeutic effectiveness shown, however, must be tempered by the fact that all patients had had extensive prior therapy, that the nature, extent, and results of such prior therapy varied tremendously, and that single-agent chemotherapy is not an optimum approach to the treatment of acute leukemia.

SWOG (4) previously carried out a similar study using doxorubicin given every 3 weeks in single doses. The overall CR rate in non-CMLBT patients is higher in the present study (22% vs 12%) than that observed with doxorubicin. However, a direct comparison of response rates is difficult. The present study was favored by a high percentage (64%) of patients having previously responded to therapy.

This figure is not available from the doxorubicin study, but it was carried out at a time when response rates were much lower. Rubidazone effects were potentially compromised in the present study, however, by prior anthracycline therapy, which was not permitted in the doxorubicin trial. At most, comparison of the two studies indicates that rubidazone is at least as effective as doxorubicin in previously treated patients.

Each of the criteria used to designate poor-risk patients has been shown in SWOG studies (unpublished data) to be associated with poor tolerance to myelosuppression. The present strategy of dose reduction was adopted in an effort to reduce the risk of fatal myelosuppression in this group. That these were indeed poor-risk patients is supported by the fact that 43% did not receive a second dose of rubidazone, either because of death or judgmental termination of antileukemia therapy. Only 40% received a dose increase. The merits of this approach can be argued but not settled by these data.

The failure of the present study to achieve the results obtained by Benjamin et al (2) at M. D. Anderson Hospital is unexplained. Forty percent of the patients in the present study were referred by M. D. Anderson investigators, and although these patients faired better than the group as a whole (five of 20 CRs in patients with AML and five of seven CRs in patients with ALL), their previous experience was not duplicated. Examination of patient characteristics, amounts of drug given, and in vitro assays of the drug failed to account for the lower response rate.

Lack of CR in patients with CMLBT is not surprising in view of the nearly universal experience of unresponsiveness to therapy in this disease. It is unfortunate that over one half of these patients were considered poor risks, thereby reducing the rubidazone dose, but review of the records revealed no evidence of significant useful activity even at full dose levels.

Toxicity of rubidazone was quite similar to that seen with doxorubicin and daunorubicin. The chief difference was the occurrence of acute febrile reactions that were often associated with generalized erythema. The potential danger of such reactions is emphasized by the death of one patient and life-threatening toxic effects in another. The incidence of mucositis was lower than might have been expected, but it was quite severe in two of the three patients in which it was reported. Although apparently not a major problem, phlebitis appeared to be more common with rubidazone than with doxorubicin. It is surprising that only one questionable case of anthracycline cardiomyopathy was observed in this large group of patients, especially in view of the fact that nearly all had prior therapy with doxorubicin. This is probably best explained on the basis of conservative dose ceilings rather than any lessened risk of cardiac damage with rubidazone or effectiveness of the ECG monitoring used.

On the basis of this positive study, SWOG developed a four-drug combination of rubidazone, vincristine, cytarabine, and prednisone. This is now being tested in a large groupwide study of newly diagnosed patients in a randomized trial with doxorubicin, vincristine, cytarabine, and prednisone.

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Results of a Phase II Protocol for Evaluation of New Chemotherapeutic Regimens in Patients With Inoperable Non-Small Cell Lung Carcinoma (EST-2575, Generation I)[1,2]

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SUMMARY

Two hundred and eighty-four patients with inoperable non-small cell lung carcinoma were randomized by the Eastern Cooperative Oncology Group to receive one of seven primary chemotherapy regimens: cyclophosphamide and methotrexate; Baker's antifol; vincristine, bleomycin, and methotrexate; melphalan; cyclophosphamide and CCNU (control arm); 5-FU and procarbazine; and hexamethylmelamine, doxorubicin, and methotrexate (HAM). Patients with disease progression were eligible for treatment with VM-26 or ascorbic acid. HAM resulted in higher response rates than cyclophosphamide and CCNU in patients with adenocarcinoma (32%) and large cell carcinoma (23%). It was not tested in patients with squamous cell carcinoma. In terms of survival, HAM was significantly better than cyclophosphamide and CCNU in patients with limited disease. Its toxicity was predominantly hematologic and gastrointestinal. This regimen is being further evaluated by the Eastern Cooperative Oncology Group in patients with inoperable non-small cell lung carcinoma. Crossover therapy with VM-26 or ascorbic acid had no therapeutic benefit.

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: The American Oncologic Hospital, The Fox Chase Center (*R. H. Creech* and *B. A. Mason*, CA-18281), and Pennsylvania Hospital (*H. J. Lerner*, CA-13613), Philadelphia, PA; Sidney Farber Cancer Institute, Boston, MA (*C. R. Mehta*, CA-23318); National Cancer Institute, Veterans Administration Medical Oncology Branch, Washington, DC (*M. Cohen*); Albany Medical College, NY (*M. Donavan* and *R. Sponzo*, CA-06594); Medical College of Ohio, Toledo (*R. T. Skeel*); Brookdale Hospital Center, Brooklyn, NY (*F. Ahmed*); Roswell Park Memorial Institute, Buffalo, NY (*P. J. Creaven*, CA-12296); and University of Rochester Cancer Center, NY (*E. Foelsch*, CA-11083). Other participating institutions include: University of Alberta, Edmonton, Canada; Albert Einstein College of Medicine, Bronx, NY (CA-14958); Baystate Medical Center, Springfield, MA (CA-20182); Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Georgetown University School of Medicine, Washington, DC (CA-02824); Hahnemann Medical College, Philadelphia, PA (CA-13611); Jackson Memorial Hospital, Miami, FL; University of Florida-Jacksonville (CA-19819); Jefferson Medical College, Philadelphia, PA (CA-14215); Lahey Clinic Foundation, Boston, MA (CA-12880); New York University Medical Center, New York (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Centre Hospitalier de Bobigny, Paris, France (CA-19273); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, PA (CA-18653); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); University of Pretoria, South Africa (CA-21692); SUNY-Stonybrook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); Wisconsin Clinical Cancer Center, Madison (CA-21115); and Yale School of Medicine, New Haven, CT (CA-15485).

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In October 1975, the Eastern Cooperative Oncology Group (ECOG) activated a phase II protocol to screen seven primary and two secondary drug regimens for antineoplastic effect in patients with inoperable non-small cell lung carcinoma. The regimens tested in this study (table 1) were chosen because they had not previously received adequate clinical trial, showed promise in early phase II trials, or were theoretically potentially active. Regimen A of Straus (1), a kinetically based combination of cyclophosphamide and methotrexate, had induced partial regressions in five of seven patients with large cell carcinoma and in five of eight patients with adenocarcinoma; all of these patients had extensive lung cancer. Regimen B (Baker's antifol), a functional analog of methotrexate, had not received an adequate phase II clinical trial. Regimen C (vincristine with delayed bleomycin and methotrexate) was based on laboratory evidence that vincristine can increase the number of tumor cells in mitosis and the G_2 phase 6 hours after administration and on a report that vincristine synchronization and bleomycin treatment resulted in a 27% response rate in 15 patients with lung cancer (2). In the summary of Blum et al (3), the bleomycin response rate was 16% in 97 evaluable patients with squamous cell lung carcinoma. Regimen D (melphalan) had not previously received an adequate trial. Regimen E (cyclophosphamide and CCNU) was used as a control arm. The ECOG (4) had previously reported response rates of 4% (complete response) and 8% (partial response) in patients with metastatic lung adenocarcinoma. Regimen F (5-FU and procarbazine) was tested because Samuels et al (5) had reported that procarbazine had induced objective regressions in three of nine patients with adenocarcinoma, in three of 13 with squamous cell carcinoma, in one of five with large cell anaplastic carcinoma, and in seven of nine with small cell lung carcinoma. However, Gottlieb and Serpick (6), using oral procarbazine and 6-hour infusions of 5-FU did not find any responses in 12 patients with advanced inoperable carcinoma of the lung. Regimen G (hexamethylmelamine, doxorubicin, and methotrexate) combined three drugs with known mild antineoplastic activity in lung cancer (7,8). Regimen H (VM-26) was used as a crossover therapy because it had not previously received an adequate trial in patients with lung carcinoma (9). Regimen I (ascorbic acid) was selected as the second crossover therapy because Cameron and Campbell (10) had tested this drug at doses of 10 g/day iv and/or orally in 50 patients with advanced cancer, seven of whom had lung carcinoma. Although no partial responses were noted in the patients with lung cancer, four had subjective benefit.

PATIENTS AND METHODS

In this protocol, seven primary and two crossover drug treatment arms were screened for activity in patients with histologically confirmed, surgically

TABLE 1.—Treatment arms (EST-2575, generation I)

Treatment arm	Drug	Dose (maximum dose)	Route	Schedule
A	Cyclophosphamide	1.1 g/m²	Iv	Every 3 wks
	Methotrexate	20 mg/m²	Orally	Days 7–21, twice a wk
B	Baker's antifol	500 mg/m²	Iv (1.5 hrs)	Once a wk
C	Vincristine*	1 mg/m² (2 mg)	Iv	Once a wk
	Bleomycin	20 units/m² (30 units)	Iv	Once a wk
	Methotrexate	40 mg/m²	Iv	Once a wk
D	Melphalan	3.5 mg/m²	Orally	Twice a day, Days 1–5 every 5 wks
E	Cyclophosphamide	700 mg/m²	Iv	Every 3 wks
	CCNU	70 mg/m²	Orally	Every 6 wks
F	5-FU	500 mg/m²	Iv	Days 1–5 every 5 wks
	Procarbazine	50→150 mg/m² (200 mg)	Orally	Days 1–14 every 5 wks
G	Hexamethylmelamine	100 mg	Orally	Daily
	Doxorubicin	40 mg/m²	Iv	Every 3 wks
	Methotrexate	40 mg/m²	Iv	Every 3 wks
H	VM-26†	67 mg/m²	Iv	Once a wk
I	Ascorbic acid	3→5 g/m²	Orally	Daily

*Given 2–6 hrs before bleomycin and methotrexate.
†Given in 150–300 ml of normal saline solution over 35 mins.

incurable squamous cell carcinoma, adenocarcinoma, or large cell carcinoma of the lung. Patients with small cell lung carcinoma were specifically excluded. All patients had to have measurable or evaluable disease, an ECOG performance status of 0–3 (bedridden patients excluded), normal marrow function (wbc count > 4000/mm³ and platelet count > 100,000/mm³), normal renal function (serum creatinine < 1.5 mg/100 ml), normal hepatic function (bilirubin < 2 mg/100 ml), no previous chemotherapy, and no prior radiation therapy to the area of evaluable or measurable disease. Patients were stratified by histologic type (squamous cell carcinoma, adenocarcinoma, and large cell anaplastic carcinoma); performance status (0–1 [ambulatory] vs 2–3 [nonambulatory]); extent of disease (limited vs extensive); and sex. All patients were randomized by the ECOG Operations Office using the above stratification characteristics. Patients entered in the crossover treatment arms were also randomized by the Operations Office.

After randomization, patients were given the protocol drugs outlined for treatments A–G in table 1. The treatment arms varied among cell types: squamous cell carcinoma (A–E), adenocarcinoma (A, B, E, F, and G), and large cell carcinoma (A, B, E, and G). Patients who developed progressive disease were eligible to receive crossover regimens H and I in table 1.

Prior to starting chemotherapy, each patient had the following evaluations: history and physical examination, performance status, tumor measurements, hemoglobin, wbc count and differential, platelet count, BUN, SGOT, bilirubin, alkaline phosphatase, calcium, and chest roentgenogram. Liver, bone, and brain scans, skeletal surveys, and bone marrow aspirations and biopsies were performed if clinically indicated. At each followup visit the patient's performance status and blood cell counts were reassessed. Tumor measurements, physical examination, chest roentgenogram, BUN, bilirubin, SGOT, alkaline phosphatase, and calcium were repeated at intervals of 3-4 weeks. Scans were repeated as clinically indicated to evaluate symptoms or response.

For each of the treatment arms, there were specific de-escalation and escalation criteria. The doses of cyclophosphamide, methotrexate, Baker's antifol, melphalan, CCNU, 5-FU, doxorubicin, and VM-26 were decreased according to the degree of hematologic toxicity encountered. Procarbazine and ascorbic acid doses were reduced for gastrointestinal toxicity. Methotrexate and Baker's antifol had dose reductions for elevation of hepatic enzymes or serum creatinine. Bleomycin was discontinued for pulmonary toxicity presenting as cough, dyspnea, rales, or pulmonary infiltrates. Doxorubicin was discontinued at a cumulative dose of 550 mg/m². Vincristine, 5-FU, and hexamethylmelamine doses were reduced or discontinued depending on the degree of neurologic toxicity. Dose reductions were required for skin and mucosal toxicity related to methotrexate, Baker's antifol, and bleomycin.

Patients were evaluated for response, using standard ECOG criteria. A complete response was defined as the complete disappearance of all clinically detectable malignancy. A partial response for measurable disease was defined as a reduction of ≥ 50% of the product of the longest perpendicular diameters of known malignancies without a > 25% increase in the size of any area of malignancy or the appearance of new areas of malignant disease. An objective regression of evaluable, nonmeasurable lesions was defined as a decrease in tumor area of ≥ 80% that was confirmed by two independent investigators. Standard ECOG toxicity criteria (none/mild, moderate, severe, and life-threatening) were used to evaluate chemotherapy-induced side effects.

During protocol chemotherapy, local radiation therapy could be given to symptomatic areas of malignant disease. If, however, these areas were the only areas of measurable or evaluable malignancy, the patient was to be withdrawn from the study. Steroids could be used for the management of central nervous system metastases.

All patients' on-study forms, flow sheets, measurement forms, and summary and evaluation reports were reviewed by the Drug Study Chairman and by the Statistical Laboratory. If further information was necessary, the principal investigators were contacted.

RESULTS

Case Accrual and Patient Characteristics

This study was started in October 1975. Treatments C, D, and F showed negligible response and were terminated in August 1976. Treatment arms A, B, and G were terminated in August 1977. Treatment E continued to accrue patients as the control arm in a subsequent generation of EST-2575. For purposes of comparison in this protocol, only the patients randomized to treatment E between October 1975 and December 1977 were evaluated.

Three hundred and thirty patients were entered in the study. Sixteen patients were cancelled prior to the start of therapy, six were improperly entered, 20 did not meet eligibility requirements, and four were lost to followup. The remaining 284 patients served as the data base for this report.

Analysis of Response

Two hundred and eighty-three evaluable patients were analyzed. One patient was not evaluable because no tumor measurements were available after the patient received one dose of chemotherapy. Treatment E (cyclophosphamide and CCNU) was considered the standard ECOG chemotherapy arm in this study (4).

The objective tumor response by treatment is seen in table 2. The overall complete and partial response rate was 13%. Only treatment G (hexamethylmelamine, doxorubicin, and methotrexate) had a significantly higher response rate than treatment E ($P = 0.01$, two-sided Fisher's test).

In table 3 the objective response rate by treatment and histologic cell type is shown. It should be noted that treatments A, B, and E were the only arms to which all three cell types were randomized. Treatments C and D were used only in patients with squamous cell carcinoma, and treatment F was used only in patients with adenocarcinoma. Both patients with adenocarcinoma and those with large cell carcinoma received treatment G.

A linear logistic model (11) was developed to test treatment effects while simultaneously identifying and adjusting the effects of the following concomitant variables: cell type, performance status, stage of disease, and prior radiotherapy. Since the same treatments were not used in all three cell types, it was convenient to develop two different models. One model was developed to test for the prognostic significance of the three cell types. The second model was developed to verify that, after adjusting

TABLE 2.—Objective tumor response by treatment*

Treatment	Complete response	Partial response	No. responding/ No. treated (%)
A	1	7	8/61 (13)
B	1	8	9/62 (15)
C	0	2	2/20 (10)
D	0	0	0/18 (0)
E	0	5	5/62 (8)
F	0	2	2/19 (11)
G	1	10	11/41 (27)
Total	3	34	37/283 (13)

*Values = No. of patients.

TABLE 3.—Objective tumor response by treatment and cell type

Cell type	Treatment	Complete response	Partial response	No. responding/ No. treated (%)
Squamous cell carcinoma	A	1	2	3/23 (13)
	B	1	4	5/22 (23)
	C	0	2	2/20 (10)
	D	0	0	0/18 (0)
	E	0	2	2/29 (7)
	Total	2	10	12/112 (11)
Adenocarcinoma	A	0	1	1/19 (5)
	B	0	1	1/21 (5)
	E	0	2	2/23 (9)
	F	0	2	2/19 (11)
	G	1	5	6/19 (32)
	Total	1	11	12/101 (12)
Large cell carcinoma	A	0	4	4/19 (21)
	B	0	3	3/19 (16)
	E	0	1	1/10 (10)
	G	0	5	5/22 (23)
	Total	0	13	13/70 (19)

*Values = No. of patients.

for the effects of other prognostic factors, treatment G was a more active drug regimen than the standard treatment E.

The first model included the 185 patients who received treatments A, B, or E. Since these were the common arms for all three cell types, this model tested whether or not cell types significantly affected the response rate. There were no significant differences in response among the cell types, and treatments A and B were not significantly different from treatment E.

The second model was used to investigate the effect of treatment G after adjusting for cell type, performance status, stage of disease, and prior radiotherapy. The 112 patients with squamous cell carcinoma were excluded because they did not receive treatment G. Also excluded were the 19 patients with adenocarcinoma who received treatment F. The model included 152 patients with adenocarcinoma or large cell carcinoma who were randomized to treatments A, B, E, and G. The following are the main findings: (*a*) treatment G had a significantly higher response rate than treatment E (11 of 41 patients vs five of 62, respectively; two-sided test, $P = 0.01$), whereas treatments A and B did not; and (*b*) patients with limited disease who were treated with regimen G had a significantly higher response rate than those with extensive disease (five of 12 vs six of 29, respectively; two-sided test, $P = 0.01$).

Time to Response and Response Duration

The median times to response and duration of response by cell type are given in table 4. The median time to response for the 37 responders was 6.6 weeks, while the median duration of response was 17.6 weeks. The curves of response duration for the three cell types were similar. The three complete responders in this study had limited disease and a performance status of 1; two had squamous cell carcinoma and one had adenocarcinoma. The durations of complete response were 101, 126, and 433+ days. The survival times were 133, 682+, and 820+ days.

Time to Treatment Failure

The time interval from the date of randomization to the date of onset of progressive disease or the date of death was defined as the time to treatment failure. The time to treatment failure for the 283 patients evaluable for tumor response was compared among the seven primary drug regimens. The estimated median time to failure was 9 weeks for all treatments, 7.1 for patients with squamous cell carcinoma, 9.4 for patients with adenocarcinoma, and 10.5 for patients with large cell carcinoma. There were no significant differences among the three cell types or by drug regimens within each cell type.

Analysis of Survival

All 284 eligible patients were analyzed for survival. When survival curves were compared by cell type, there were no significant differences. The estimated median survival times were: 13.3 weeks, squamous cell carcinoma; 18.9, adenocarcinoma; and 26.1, large cell carcinoma. The estimated median survival times by treatment regimen have been summarized in table 5. By using the log-rank test, the following concomitant variables appeared to be highly prognostic for survival: (*a*) initial performance status (ambulatory patients lived longer, $P = 0.0004$), (*b*) stage of disease (patients with limited disease lived longer, $P = 0.0095$), (*c*) prior weight loss (patients with $< 5\%$ weight loss lived longer, $P = 0.003$), and (*d*) prior surgery (patients who had had surgery lived longer, $P = 0.04$).

To obviate any imbalances among the seven different treatment arms with respect to cell type, stage of disease, and performance status, the simultaneous effects of treatment, cell type, and other variables on patient survival were analyzed according to the modeling technique of Cox (12). Patients with squamous cell carcinoma were excluded from this model because none received treatment G. Patients with adenocarcinoma who received treatment F were also excluded. The

TABLE 4.—Median time to response and duration of response by cell type

Cell type	No. of patients responding	Median time to response (wks)	Median duration of response (wks)
Squamous cell carcinoma	12	6.0	17.1
Adenocarcinoma	12	8.8	16.0
Large cell carcinoma	13	3.7	15.1
Total	37	6.6	17.6

TABLE 5.—Survival by treatment and stage of disease

Treatment	Estimated median survival time (wks)	Stage of disease*	Survival rates (%)		
			6 mos	12 mos	18 mos
A	23.3	L	64	31	2
		E	41	8	0
B	19.9	L	41	2	0
		E	41	6	0
C	12.6	L/E			
D	12.9	L/E			
E	15.9	L	29	15	0
		E	27	13	5
F	16.0	L/E			
G	33.9	L	70	46	35
		E	53	21	4

*L = limited, E = extensive.

remaining 152 patients with adenocarcinoma or large cell carcinoma who were randomized to treatments A, B, E, and G were divided into two groups according to the extent of disease (limited vs extensive). The survival rates at 6, 12, and 18 months have been tabulated in table 5. A separate model was developed for each group because preliminary analyses revealed the presence of an interaction between treatment G and extent of disease. The following conclusions were made.

Patients with limited disease.—(*a*) Treatment G resulted in significantly longer survival than treatment E (two-sided test, $P = 0.02$); (*b*) ambulatory patients lived significantly longer than nonambulatory patients (two-sided test, $P = 0.02$); (*c*) a prior weight loss of 5%–10% resulted in significantly shorter survival than a prior weight loss of < 5% (two-sided test, $P = 0.03$); and (*d*) patients who had had prior surgery lived significantly longer than patients who had had no prior surgery (two-sided test, $P = 0.01$).

Patients with extensive disease.—Prior weight loss was the only significant prognostic variable in this category. A prior weight loss of 5%–10% resulted in significantly shorter survival than a prior weight loss of < 5% (two-sided test, $P = 0.006$). There were no significant differences in survival among the treatment arms.

Analysis of Toxicity

Complications in patients were evaluated by cause (related or unrelated to treatment), by type (hematologic, infection, bleeding, neurologic, respiratory, genitourinary, hepatic, other gastrointestinal, and skin and mucous membrane), and by degree (on a 4-point scale ranging from no toxicity to life-threatening toxicity), according to ECOG toxicity criteria. In table 6 the most severe toxic effects of each treatment arm have been tabulated. The most frequent life-threatening toxicity was hematologic. Treatments A, C, and F appeared to be more toxic than the other treatments. In this study there were 16 instances of life-threatening toxicity and three drug deaths. The toxic effects experienced by the 41 patients receiving treatment G can be seen in table 7.

The incidence of severe or worse toxicity ranged between 30% and 50% for all levels of prognostic factors. A similar comparison was made between responders and nonresponders. There was a 52% incidence of severe or worse toxicity among the 37 responders, compared to a 35% incidence among the 246 nonresponders. This difference was not statistically significant.

Crossover Therapies

When patients randomized to generations I and II of EST-2575 had disease progression, they could

TABLE 6.—Most severe toxic effects by treatment arm*

Treatment	No. treated	Hematologic		Neurologic		Respiratory	
		LT	DD	LT	DD	LT	DD
A	61	8 (13%)	1 (2%)				
B	63			1 (2%)		1 (2%)	
C	20	3 (15%)					
D	18	1 (6%)					
E	62	1 (2%)					
F	19	1 (5%)	2 (11%)				
G	41						

*LT = life-threatening, DD = drug death, values = No. of patients.

TABLE 7.—Toxic effects experienced by 41 patients receiving treatment G*

Type	Moderate (%)	Severe (%)
Hematologic	9 (22)	1 (2)
Infection	1 (2)	0 (0)
Bleeding	0 (0)	0 (0)
Neurologic	1 (2)	1 (2)
Vomiting	11 (27)	4 (10)
Other GI	4 (10)	3 (7)
Skin and mucous membrane	0 (0)	1 (3)

*Values = No. of patients.

be randomized between VM-26 and ascorbic acid. These two crossover therapies were discontinued in December 1977. One hundred and two patients were assigned to the crossover therapies. Twenty-four patients were cancelled, improperly entered, ineligible, or had no followup information. The remaining 78 patients were evaluable for this phase of the study. Two of the 38 evaluable patients treated with VM-26 had a partial response while none of the 40 evaluable patients receiving ascorbic acid had a partial response. Neither of the VM-26 patients had responded to earlier therapy. There was no significant difference in survival time between the two treatment arms. The median survival time with VM-26 was estimated at 13.9 weeks while the median survival time with ascorbic acid was estimated at 12.9 weeks. The two survival curves were similar. The two regimens differed significantly in their incidence of severe or worse toxicity (two-sided test, $P = 0.01$). VM-26 was the more toxic regimen; toxic effects were severe in seven patients and life-threatening in one, among 38 evaluable patients. One patient who was receiving ascorbic acid had severe toxic effects.

DISCUSSION

The primary objective of this study was to screen seven primary and two secondary drug regimens for antineoplastic activity in patients with inoperable non-small cell bronchogenic carcinoma. Cyclophosphamide and CCNU (treatment E), which previously had been studied by the ECOG (4), was considered to be the standard therapy for these patients. This study showed that there were no significant differences between cyclophosphamide plus CCNU and five of the other six drug regimens, with respect to tumor response and survival.

The kinetically based combination of cyclophosphamide and methotrexate (treatment A) was not as effective in this cooperative group trial as in Straus' series (13). Straus reported a 56% objective response rate in both adenocarcinoma and large cell carcinoma of the lung, whereas in this ECOG study the response rates were 5% and 21%, respectively. The median survival times of these two cell types in ECOG patients were approximately one half those of Straus. The response rates in patients with squamous cell carcinoma were similar in both series (14%, Straus; 13% ECOG), but the median survival times differed (36 weeks, Straus; 9.3, ECOG). In this ECOG series, there were frequent deviations from suggested dose adjustments because of the complex nature of the drug escalation and de-escalation requirements.

It seems unlikely that the 20%–30% discrepancy between the ideal and the actual cyclophosphamide doses administered in this ECOG trial fully explains the differences in response rates and median survival times between these two series. Further evaluation of this regimen, particularly in large cell carcinoma, should be considered.

Baker's antifol (treatment B) deserves further evaluation in squamous cell carcinoma (five responses among 22 patients, 23%) and large cell carcinoma (three responses among 19 patients, 16%). The response rates were ≤ 11% with vincristine, bleomycin, and methotrexate (treatment C) and with melphalan (treatment D) in squamous cell carcinoma; with 5-FU and procarbazine (treatment F) in adenocarcinoma; and with cyclophosphamide and CCNU (treatment E), VM-26 (treatment H), and ascorbic acid (treatment I) in squamous cell carcinoma, large cell carcinoma, and adenocarcinoma of the lung. This obviates further evaluation of these regimens.

Hexamethylmelamine, doxorubicin, and methotrexate (HAM) was superior to the control arm of cyclophosphamide and CCNU for the treatment of inoperable adenocarcinoma and large cell carcinoma of the lung, with respect to tumor response, survival in patients with limited disease, and toxicity. Because of the apparent antineoplastic activity of this regimen, HAM has been incorporated into a subsequent ECOG study of patients with inoperable non-small cell lung cancer.

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Phase I Evaluation of IMPY in a Twice Weekly Schedule[1,2]

Charles D. Haas, Usha Joseph, Robert J. Belt,* and Barth Hoogstraten[3]

SUMMARY

IMPY was given to 25 patients with advanced cancer on a twice weekly schedule in escalating doses from 165 to 3000 mg/m². Nausea, vomiting, fatigue, generalized weakness, and decreases in performance status were dose-limiting. In one patient treated at a dose of 3000 mg/m² for three doses, hemolytic anemia resulted, with a 6-g/dl decrease in hemoglobin. No tumor regressions occurred. A reasonable starting dose for phase II studies is 1200 mg/m² twice weekly for 3 weeks, with planned rapid escalation to 1800 mg/m² in patients tolerating the lower dose level.

[Cancer Treat Rep 65:439–441, 1981]

IMPY is an inhibitor of ribonucleotide reductase and reversibly inhibits DNA synthesis (1).[4] RNA and protein syntheses are not significantly inhibited (1,2).[5,6]

IMPY was selected for human trials based on moderate activity against L1210 leukemia and marginal activity against Adenocarcinoma 755, B16 melanoma, Ridgway osteogenic sarcoma, and mouse colon 38.[4,7]

In large animals, anemia was the most severe hematologic toxicity seen and is thought to be due to a direct toxic effect on the circulating erythrocytes. At higher dose levels bone marrow aplasia and hemorrhagic necrosis were noted in several organ systems.[4]

Preclinical studies in animal tumor systems demonstrated definite schedule dependence, with optimal antitumor activity resulting from three injections at 4-day intervals or two injections at 8-day intervals.[4] The twice weekly schedule was selected for our phase I study because hydroxyurea, which has a mechanism of action similar to IMPY, is frequently administered by this schedule and because of the above-mentioned superiority of intermittent scheduling.

MATERIALS AND METHODS

All patients had histologically confirmed malignant disease not curable by surgery, radiotherapy, and/or established standard chemotherapy and had an estimated survival of at least 8 weeks. No patient had received prior radiotherapy or chemotherapy within 3 weeks (6 weeks for mitomycin C or a nitrosourea) of receiving IMPY. All patients had a wbc count $\geq 4000/mm^3$, a platelet count $\geq 100,000/mm^3$, a hemoglobin concentration of at least 10 g/dl, and adequate renal (serum creatinine < 2 mg/dl) and hepatic functions (serum bilirubin, SGOT, and alkaline phosphatase $< 50\%$ greater than normal).

There were ten patients with colon cancer; 11 with lung cancer (four with squamous cell, two with

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[3]Veterans Administration Medical Center, Kansas City, MO (*C. D. Haas* and *R. J. Belt*), and Division of Clinical Oncology, Department of Medicine, University of Kansas Medical Center, Kansas City (*U. Joseph, R. J. Belt,* and *B. Hoogstraten*).

[4]Clinical brochure: pyrazolo-imidazole (IMPY) NSC 51143. Prepared by the National Cancer Institute, Bethesda, MD, Aug 1977.

[5]Progress Report 1. Biochemical and molecular biological characterization of antitumor drugs. Southern Research Institute, Birmingham, AL, SORI-KM-74-354, Nov 1974, pp 18–37. Contract N01-CM-43734.

[6]Progress Report 2. Biochemical and molecular biological characterization of antitumor drugs. Southern Research Institute, Birmingham, AL, SORI-KM-75-102, Mar 1975, pp 19–24. Contract N01-CM-43784.

[7]Progress Report 3. Biochemical and molecular biological characterization of antitumor drugs. Southern Research Institute, Birmingham, AL, SORI-KM-75-359, July 1975, pp 16–20. Contract N01-CM-43784.

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TABLE 1.—IMPY dose level vs toxicity

Dose (mg/m^2)	No. of patients/ total No. of courses	Degree of nausea and vomiting (No. of patients)	Drop in hemoglobin concentration (No. of patients)	Degree of malaise (No. of patients)
165	2/2	None	None	Severe (1) Mild (1)
330	3/3	None	None	Mild (3)
500	5/5	None	2 g/dl (1)	Mild (1) Moderate (4)
650	4/4	None	None	Mild (3) Moderate (1)
800	7/7	Mild (1) None (6)	3 g/dl (1)	Mild (1) Moderate (5) Severe (1)
1200	5/13	None (3) Mild (1) Moderate (1)	None	Mild (2) Moderate (1) Severe (2)
1600	1/0.5	Moderate	None	Severe
1700	1/0	Severe	None	Severe
1800	4/4	None (1) Mild (1) Moderate (1) Severe (1)	3 g/dl (1)	Moderate (2) Severe (2)
3000	3/1.5	Moderate (2) Severe (1)	6 g/dl (1)	Severe (3)

large cell, two with adenocarcinoma, two with poorly differentiated, and one with alveolar cell); one each with renal cell cancer, metastatic melanoma, and metastatic squamous cell carcinoma (primary site unknown); and one with both colon cancer and squamous cell cancer of the tongue.

Pretreatment evaluation included a history and physical examination, weight, performance status, tumor measurement, chest roentgenogram, ECG, cbc with differential and platelet counts, reticulocyte count, urinalysis, serum haptoglobin, rbc fragility, and SMA-12 (including serum BUN, creatinine, uric acid, total and direct bilirubin, SGOT, LDH, and alkaline phosphatase). Cbcs with differential and platelet count were done twice weekly and SMA-12 and urinalysis were repeated weekly. Other laboratory tests were repeated every 3 weeks along with clinical evaluation of disease response. When indicated, detailed evaluation of possible drug-related toxicity, such as hemolytic anemia, was undertaken.

IMPY was supplied by the National Cancer Institute, Bethesda, MD, in sterile vials containing 500 mg. The drug was reconstituted immediately before administration with 9.6 ml of sterile water for a final drug concentration of 50 mg/ml and was given as a 15-minute rapid infusion through a free-flowing iv line. IMPY was administered twice weekly for 3 weeks (one course) provided that excessive toxicity was not present. Subsequent 3-week courses were given if the patient did not have progressive disease or residual toxicity after a 1–2-week rest period.

The starting dose was 165 mg/m^2 twice weekly for 3 weeks in two patients. Subsequent dose escalations are shown in table 1 and were carried out if no toxicity was observed at the lower dose level. A minimum of three patients were treated at most dose levels. Individual patients received dose escalations after nontoxic drug courses. Once toxicity was encountered patients were maintained at this same dose if more than one course was administered. Responses are shown in table 1.

RESULTS

A total of 25 patients were entered in the study; 22 and 16 patients were evaluable for toxicity and response, respectively. Nine of the 25 patients were not evaluable for response because the study was terminated early for various reasons: five patients refused further therapy after one to three doses due to drug-induced side effects, two patients had tumor-related deaths after one dose, one patient had

rapid disease progression after receiving only one dose, and one patient developed severe hemolytic anemia after three doses.

Dose-limiting toxic effects were subjective in all patients except one and included nausea and vomiting of 12–24 hours' duration, malaise, fatigue, weakness, and anorexia which often lasted 48–96 hours after each dose. The duration of these symptoms while on a twice weekly treatment schedule often resulted in patients feeling ill and eating little or nothing for the entire treatment course of 3 weeks. Not surprisingly, patient compliance suffered, particularly at higher dose levels. There was considerable variation in patient tolerance which is best related to the pretreatment performance status (PS). Of ten evaluable courses begun at $\geq$ 1200 mg/m^2 in patients with a PS of 40–60 (Karnofsky scale), all four patients refused further therapy. In contrast, of 18 evaluable courses at the same dose in patients with a PS of 70–90, only one refused further therapy. It thus appears that high doses of IMPY produce an intolerable burden for patients whose cancers have already caused serious limitations of activity. It was not uncommon to have compliant patients request that treatment be deferred after four or five doses to allow recovery of energy and appetite prior to resuming treatment. Prior chemotherapy or radiation therapy appeared to have no influence on drug toxicity.

Objective toxic effects attributable to IMPY were uncommon. A single episode of documented hemolytic anemia occurred in a patient who received three doses at 3000 mg/m^2. This patient experienced a decrease in hemoglobin concentration of 6 g/dl associated with low haptoglobin level, elevated serum free hemoglobin, mild elevation of serum total bilirubin to 1.6 mg/dl, and reticulocytosis (corrected) of 7.8%. The patient was taken off IMPY and recovered without further complications.

A second patient receiving a dose of 1800 mg/m^2 for one course had a 3-g/dl decrease in hemoglobin concentration without demonstrable bleeding. Laboratory evaluation, however, failed to reveal evidence of intravascular hemolysis. Two other patients receiving a dose of 1800 mg/m^2 for five and two courses had a $<$ 3-g/dl decrease in hemoglobin concentration from overt tumor-related bleeding. One of five patients at a dose of 800 mg/m^2 demonstrated a 3-g/dl decrease in hemoglobin concentration from tumor-related upper gastrointestinal bleeding. Routine testing of pretreatment and posttreatment serum haptoglobin, serum-free hemoglobin, and rbc fragilities were nonproductive.

IMPY did not produce leukopenia or thrombocytopenia in any patient. Other toxic effects associated with IMPY included mild mucositis (one patient), transient hiccoughs (two), and mild transient diarrhea (three). There was no evidence of hepatic or renal damage due to the drug. One patient developed a skin rash after receiving one course at 800 mg/m^2 and was removed from the study. One patient developed severe, protracted hiccoughs after receiving one dose at 1600 mg/m^2 and refused further drug treatments. Two patients developed severe nausea, vomiting, and malaise after receiving 1.5 courses at 3000 mg/m^2 and refused further drug treatments. The few patients who showed elevated serum alkaline phosphatase and/or SGOT had objective evidence of progression of their liver metastasis.

No responses were observed among the 16 patients who completed one or more courses. Two of five evaluable patients with colorectal cancer had disease stabilization for 113 and 581+ days. Two of eight patients with non-small cell lung cancer had brief stabilization of their disease for 53 and 57 days, respectively.

DISCUSSION

Protracted malaise, weakness, nausea, and anorexia result in frequent requests for treatment delays or in noncompliance, particularly in patients with a low (40–60) PS treated at doses of $\geq$ 1200 mg/m^2. Such patients may tolerate a dose of 800 mg/m^2 on the twice weekly schedule. Patients with a PS of $\geq$ 70 can be started at 1200 mg/m^2 twice weekly for 3 weeks with escalation if well-tolerated. At a dose of 1800 mg/m^2 anemia was seen in one of three evaluable patients and subjective toxicity was marginally tolerable. Doses of 3000 mg/m^2 produce hemolytic anemia and/or noncompliance and must be regarded as excessive. Skin rash, mucositis, hiccoughing, and diarrhea occur infrequently in patients receiving IMPY. No myelosuppression or other major organ system toxic effects were observed.

We observed no tumor regressions in this group of patients with commonly refractory cancers; most had received prior chemotherapy. Prolonged disease stabilization (581 days) in one patient with colorectal cancer treated at 800 mg/m^2, then escalated and maintained at 1200 mg/m^2, is of interest. A phase II trial in this and other tumors might be considered if dose adjustments are made for PS.

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Phase II Evaluation of VP-16-213 in Patients With Advanced Breast Cancer: A Southwest Oncology Group Study[1,2]

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VP-16-213 is a semisynthetic antineoplastic product derived from podophyllotoxin. Its main antitumor effects are related both to the inhibition of mitosis by interfering with the assembly of the microtubules in the mitotic apparatus and to the degradation of intracellular DNA (2). VP-16-213 has an antitumor effect in experimental rodent tumors including Sarcomas 37 and 180 and the L1210 leukemia systems.[4,5] Nissen et al (3) observed antitumor effects with VP-16-213 in patients with Hodgkin's disease, reticulum cell sarcoma, and lymphosarcoma during a phase I study of this agent. A phase II study of the oral administration of this drug in advanced breast cancer produced three partial remissions and one improvement among 20 patients (4). The trial we are reporting was begun to evaluate the efficacy of parenteral VP-16-213 in patients with drug-resistant advanced adenocarcinoma of the breast, using a 5-day schedule of slow iv infusion.

MATERIALS AND METHODS

One hundred and nineteen patients with advanced breast cancer were entered in the study. Initially, VP-16-213 at a dose of 45 mg/m²/day × 5 was administered to 60 patients. The calculated dose was dissolved in 120 ml of normal saline, and the solution was infused iv for a minimal period of 30 minutes. The course was repeated every 21 days. After no toxicity was seen with this dose level, a pilot study was performed which demonstrated that the drug could, with only moderate toxicity, be given at a level of 75 mg/m²/day × 5 to good-risk patients. The low dose was continued until the completion of the pilot study. Fifty-nine patients received this higher dose regimen. One hundred and nineteen patients entered in this study were considered fully evaluable for response.

Most of the patients were postmenopausal (median age, 54 years; range, 26–79) and had a performance status of 2 or 3 (88% of the 45-mg/m² group and 71% of the 75-mg/m² group). Seventy-five percent of the patients exhibited multiple sites of involvement. Bone was the most commonly involved organ. All patients had previously been treated with multiple chemotherapeutic agents. Tumor measurements were determined before initiation of the study and prior to each subsequent course of therapy.

A complete remission (CR) was defined as the disappearance of all measurable evidence of tumor for a minimum of 4 weeks. A partial remission (PR) was defined as a ≥ 50% reduction in the sum of the products of the diameters of measurable lesions, provided no lesions increased in size, no new lesions developed, and the remission lasted for a minimum of 4 weeks. Stable disease was defined as an improvement of measured lesions for at least 4 weeks, but less than a PR, or a steady state that lasted for at least 8 weeks (no further increase in previously progressing disease after initiation of therapy). Increasing disease was defined as an unequivocal increase of at least 25% in the size of any measured

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[3]We thank Lynn Barylski, Gerri Socall, and Betty Chinn for clerical assistance.

[4]Handelsman H. Goldsmith MA, and Slavik M. Clinical brochure: 4'-demethyl-epipodophyllotoxin-β-D-ethylidene glucoside (NSC-141540) VP 16-213. Prepared by the National Cancer Institute, Bethesda, MD, Jan 1973.

[5]Sandoz Pharmaceuticals. Preclinical brochure: VP-16-213. East Hanover, NJ, 1970.

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TABLE 1.—Toxicity of VP-16-213 at a dose of 75 mg/m²

Toxic effect	No. of patients with grade*—				
	0	1	2	3	4
Leukopenia	10	8	10	11	8
Thrombocytopenia	25	7	6	5	4
Anemia	38	2	6	1	0
Nausea and vomiting	32	10	3	1	1
Alopecia	39	1	1	4	2

*Definitions of grades appear in the Southwest Oncology Group Administrative Manual.

lesion, the appearance of a new lesion, uncontrolled hypercalcemia, or clearly progressive skeletal involvement. The duration of remission or stable disease was determined from the start of therapy until progressive disease (25% increase in the size of the lesion) became evident.

VP-16-213 was supplied by the National Cancer Institute (Bethesda, MD) in vials containing 100 mg of the drug dissolved in a combination of organic solvents which include 80 mg of Tween 80, 600 mg of polyethylene glycol 300, 30 mg of benzyl alcohol, 2 mg of citric acid (anhydrous), and 1 mg of absolute alcohol. The dose of VP-16-213 was diluted in normal saline and was infused iv for 30–60 minutes. Patients who received a dose of 75 mg/m² of VP-16-213 received an escalation of one dose level if there was no leukopenia and/or thrombocytopenia. The drug dose was reduced by one level if the wbc count was between 1.0 and 2.0 × 10^3/mm³ or if the platelet count was between 50 and 75 × 10^3/mm³. The drug was reduced by two levels if the wbc count was < 1.0 × 10^3/mm³ and/or the platelet count was < 50 × 10^3/mm³.

RESULTS

All 119 eligible patients were considered evaluable in this study. Eight patients (7%) experienced tumor reduction. Three patients (1%) achieved a CR or PR with the 45-mg/m² dose. Five patients (9%) had a PR with the 75-mg/m² dose. Forty-six patients had stabilization of their disease for 8 weeks. The only CR was achieved with the lower dose, 45 mg/m². However, proportionately more tumor reductions were seen in those patients treated with the higher dose, suggesting that the higher dose was more cytotoxic.

Toxicity data are available for 47 patients who were treated with 75 mg/m² of VP-16-213. Myelosuppression was the limiting toxic effect (table 1). More leukopenia was experienced than thrombocytopenia. Forty percent of those patients who received this dose of VP-16-213 had grade 3 or 4 leukopenia; eight patients had life-threatening leukopenia. Nineteen percent of the patients who received this dose of VP-16-213 experienced grade 3 or 4 thrombocytopenia; four of these patients had life-threatening thrombocytopenia. Nausea and vomiting were mild and controllable. There were no drug-related deaths in this study.

DISCUSSION

The response of breast cancer to VP-16-213 was more impressive than it would first appear because these patients had been heavily treated and had far-advanced disease. Many patients were preterminal. Patients such as these are usually resistant to any therapy. VP-16-213 may be expected to produce more CRs and PRs if it is used to treat early recurrent breast cancer.

Our results are similar to those of Ahmann et al (5) who, using an alternating-day dose schedule, observed stability of disseminated breast cancer in seven of 19 patients. The duration of stability was 15.6 weeks in their study compared to 20 weeks in our study. Similar results were also seen by Falkson et al (4) who used the oral route of administration. The toxicity of VP-16-213 at a dose of 75 mg/m²/day × 5 in this study is tolerable. The minimal activity in breast cancer with mild toxicity suggests that VP-16-213 should be evaluated in early disseminated disease and also in combination with other active agents.

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Study of Tamoxifen in Metastatic Renal Cell Carcinoma and the Influence of Certain Prognostic Factors: A Southwest Oncology Group Study[1,2,3]

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SUMMARY

A prospective study of tamoxifen therapy in doses of 10 mg twice daily was carried out in 79 patients with advanced renal cell cancer. The objective response rate (complete response plus partial response) was 6.3% (five of 79 patients), while 34.1% (27 of 79 patients) had stable disease. Survival of this group of patients (complete response plus partial response plus stable disease) was significantly longer than that of patients with progressive disease ($P < 0.04$). Also, among patients with a good performance status at the beginning of the study, survival was longer than among those with a poor performance status ($P < 0.001$). No statistical significance was found in the survival of these patients according to sex ($P = 0.55$) or whether or not they had received previous systemic therapy ($P = 0.97$). Toxicity was low and acceptable. We concluded that stratification according to performance status may be used in future randomized systemic therapy protocols for patients with metastatic renal cell cancer. Also, in spite of the low response rate to hormonal therapy including tamoxifen, the use of these agents with combination chemotherapy may enhance the overall response rate without increasing side effects.

[Cancer Treat Rep 65:447–451, 1981]

The possible endocrine dependency of some renal cell cancers has been known for many years, and efforts have been made to treat advanced and recurrent disease with hormones, chiefly with medroxyprogesterone acetate (Provera) and testosterone propionate (1–6). Of 272 collected cases from various investigators, reported by Bloom (6), the overall objective response rate was 15% (range, 6%–33%).

In 1967, Bloom et al (7) reported that the estrogen antagonist agent (nafoxidine hydrochloride) given to hamsters inhibited the growth of transplanted estrogen-induced renal tumors. Survival of treated animals was prolonged compared with that of the control group.

Tamoxifen, an estrogen antagonist, has not been associated with the severe skin photosensitivity reported with nafoxidine (8,9) and is currently widely used in patients with metastatic breast cancer (10–13).

The efficacy of tamoxifen therapy in patients with disseminated renal cell cancer was tested in a prospective study by the Southwest Oncology Group.

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[3]Tamoxifen was supplied by ICI Americas, Inc, Wilmington, DE.

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METHODS AND MATERIALS

Patients with histologically proven disseminated renal cell carcinoma who had not received prior antiestrogen agents were entered in this study. All patients had measurable disease which was no longer amenable to surgery or radiotherapy. Patients entered in the study had an expected survival of at least 8 weeks. Patients must not have received previous chemotherapy or hormonal therapy for 4 weeks and had to have clearly progressive disease prior to entering this study. Radiotherapy to the pilot lesion must have been completed 3 weeks prior to entry. Bone lesions which had received radiotherapy at any time were not eligible as measurable disease to be followed. Tamoxifen was given at a dose of 10 mg twice a day orally for at least 6 weeks. If an objective remission was obtained, treatment was continued until disease progression occurred. If the lesion(s) was static, therapy was to be continued but re-evaluated at 6-week intervals until objective remission or disease progression was documented. When definite tumor progression occurred, tamoxifen was stopped.

Before entry in the study, all patients had a complete history and physical examination, and performance status, documentation of all measurable lesions, and histologic types were recorded. Cbcs with leukocyte differential and platelet counts were performed before the start of tamoxifen, on Day 10, and every 6 weeks while receiving therapy.

Serum creatinine, BUN, uric acid, alkaline phosphatase, SGOT, LDH, bilirubin, calcium, and electrolytes were done before treatment and every 6 weeks while receiving tamoxifen. Chest roentgenogram, iv pyelogram, bone survey, and bone and liver scans were performed before the start of therapy and repeated at every 3–6 weeks to evaluate tumor response, if indicated.

Objective tumor response was defined as follows:

Complete remission (CR)—the complete disappearance of all clinical evidence of tumor, without the appearance of new lesions for a minimum of 4 weeks. In patients with bone disease, all lytic lesion(s) must have remineralized. No bone pain should be present (without analgesic medication). Remodeling of previously distorted skeletal structure must be evident.

Partial remission (PR)—a $\geq$ 50% decrease in the sum of the products of perpendicular diameters of all measurable lesions. Patients with liver metastasis had to have $\geq$ 30% reduction in the sums of measurements below the costal margin. No simultaneous increase in the size of any lesion or the appearance of any new lesions may occur.

Stable disease—steady state or less than both PR and disease progression. There may be no appearance of new lesions and no worsening of the symptoms.

Progressive disease—unequivocal increase by at least 50% in the size of any measurable lesions, the appearance of new lesions, uncontrolled hypercalcemia, or clearly progressive skeletal involvement manifested by an increasing number of lytic lesions.

RESULTS

Seventy-nine patients were entered in this study and were evaluated for this report. There were 61 males and 18 females 26–81 years of age (median, 58). Sixty-two patients had previously undergone nephrectomy, while in 17 patients the histologic confirmation of renal cell cancer was done by biopsy of metastatic lesions, with an iv pyelogram and renal arteriogram confirming the primary site. Twenty-seven patients had had previous radiotherapy either to the kidney area or to areas of metastatic disease as palliative treatment. Forty-six patients had had no previous systemic therapy (hormonal therapy or chemotherapy), 12 had had hormonal therapy only (progestational agent), five had had chemotherapy only, and 14 had had combined chemotherapy and hormonal therapy before entry in the study. For two patients such information was not available. The sites of metastatic disease are shown in table 1. Thirty-nine of the patients (49%) had a single metastatic site and 40 had multiple sites.

Two patients achieved CRs lasting 24 and 27 weeks and three achieved PRs lasting 13, 18, and 55 weeks. The responding lesions included: bone (two patients) and lung, skin, and nodes (one patient each). Twenty-seven patients had stable or improved disease, less than PR. The median survival of responding patients and those with stable disease (32 patients) was 54 weeks (range, 10+–72+). Forty-seven patients (59.5%) had definite progressive disease while receiving tamoxifen. Their median survival was 13 weeks, with a range of 2–56+ weeks. This difference is statistically significant ($P < 0.04$) (fig 1). Survival according to sex, prior sys-

TABLE 1.—Incidence of metastatic disease in patients with advanced renal cell cancer

Site	No. of patients (%)
Lung	59 (74.7)
Bone	30 (38.0)
Liver	13 (16.5)
Nodes	9 (11.4)
Brain	5 (6.3)
Skin	5 (6.3)
Other	10 (12.7)

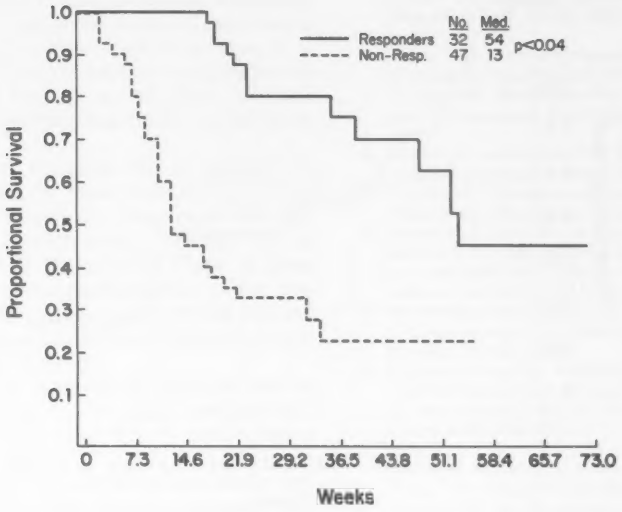

FIGURE 1.—Survival (wks) of responders + patients with stable disease vs nonresponders.

temic therapy, and performance status at the start of therapy was evaluated.

The survival range for males was 2–72+ weeks (median, 32) and for females was 5+–56+ weeks (median, 36+) ($P = 0.55$). Patients with no prior hormonal or systemic chemotherapy had a survival range of 2–72+ weeks, with a median of 32 weeks. Patients with prior hormonal therapy and/or chem-

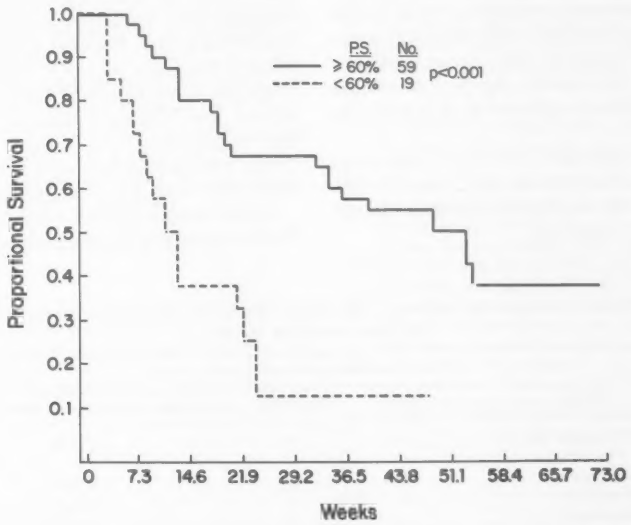

FIGURE 2.—Survival (wks) of patients with advanced renal cell cancer according to performance status.

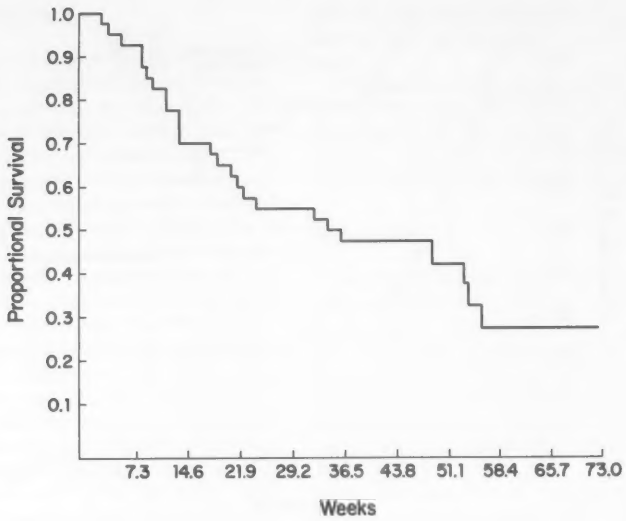

FIGURE 3.—Overall survival (wks) of 79 patients with advanced renal cell cancer.

otherapy had a survival range of 3–56+ weeks, with a median of 36 weeks ($P = 0.97$). There was a significant difference in survival according to performance status regardless of the response to tamoxifen. Patients with a performance score of $\geq 60\%$ (59 patients) had a median survival of 48 weeks as compared to 13 weeks for patients with a poor performance status ($< 60\%$) ($P < 0.001$) (fig 2). The overall survival of all the patients entered in this study is shown in figure 3.

The incidence of toxicity and its severity was very low and acceptable. Sixty-three patients (79.7%) experienced no side effects. Table 2 shows the types of tamoxifen toxicity reported.

DISCUSSION

The previously reported response rates of patients with metastatic renal cell cancer to hormonal therapy with medroxyprogesterone acetate or testosterone is low. Several series reported after 1971 show a response rate of $< 10\%$ compared to higher response rates reported before 1971 (1–6). In spite of the low response rate to progesterone, these agents had been used in many combinations for patients with advanced renal cell cancer, with higher response rates reported (14–17).

Antiestrogen agents have been shown to be effective against transplanted estrogen-induced renal

TABLE 2.—Types and severity of side effects due to tamoxifen in patients with advanced renal cell cancer

Type	No. of patients in which toxicity was—			Total No. of patients (%)
	Mild	Moderate	Severe	
Nausea and/or vomiting	3	2	2	7 (8.9)
Leukopenia	2	1	2	5 (6.3)
Weakness	1	1		2 (2.5)
Anorexia	1	1		2 (2.5)
Flushing	1			1 (1.3)
Abdominal pain	1			1 (1.3)
Sweating	1			1 (1.3)

tumors in hamsters (7) and occasional responses have been seen with advanced renal cell carcinomas in man (18,19). In 20 evaluable patients, Feun et al (19) reported one PR and one patient with improved disease (both lung lesions and no prior systemic therapy), and six patients had stable disease with nafoxidine therapy.

In this Southwest Oncology Group prospective study with tamoxifen in doses of 20 mg/day in patients with advanced and recurrent renal cell cancer, the objective response rate (CR plus PR) of 6.3% was low (five of 79 patients). Three of these patients had had previous chemotherapy and/or hormonal therapy. Twenty-seven patients (34.1%) had either stable or improved disease. Thirteen of them had had no previous therapy.

The response rate to hormonal therapy may be increased by selecting and treating those patients with positive estrogen receptors in tumor tissue (20).

The large number of patients in this study permitted us to evaluate survival in responders and nonresponders, and to look at possible prognostic factors like sex, previous therapy, and performance status. Statistically significant improved survival was observed in responders (CR plus PR plus stable disease) and patients with a good performance status, as compared to nonresponding patients and those with a poor performance status (figs 1 and 2).

Several side effects of tamoxifen have been reported in patients with breast cancer undergoing treatment although several manifestations of the disease itself have been attributed to tamoxifen as a measure of caution (10–13). Side effects were generally mild, consisting of hot flushes, transient leukopenia and thrombocytopenia, nausea, fluid retention, skin rash, and tumor pain. As with other studies (10–13), it is difficult to be certain if the side effects reported in this study (table 2) are due to tamoxifen or the underlying disease.

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Cyclophosphamide and VP-16-213 With or Without Cisplatin in Squamous Cell and Small Cell Lung Cancers[1,2]

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SUMMARY

Forty-seven patients with squamous cell or small cell lung cancer (28 [60%] of whom had cardiac disease too severe to permit the use of doxorubicin) were treated with cyclophosphamide and VP-16-213 with or without cisplatin. Four of 11 patients with squamous cell (36%) and 19 of 24 patients with small cell (79%) cancers had tumor regressions (eight complete regressions) on the three-drug regimen. Median survival times for the 31 patients with small cell lung cancer were 9 months for those with extensive disease and 12.2 months for those with limited disease. The data suggest that the roles of VP-16-213 and cisplatin need further evaluation in these cell types and that doxorubicin may not be mandatory for successful treatment of squamous cell and small cell lung cancers.

[Cancer Treat Rep 65:453–458, 1981]

VP-16-213 has been shown (1,2) to have significant activity in small cell lung cancer (SMCLC). VP-16-213 combined with cyclophosphamide, another agent with significant activity in SMCLC (3), produced very encouraging results in a small number of patients (4). We have also demonstrated that VP-16-213 has activity against squamous cell lung cancer (SQCLC), producing regressions in five of 20 patients (5). In 1976, Gale et al (6) demonstrated synergism from the combined use of cyclophosphamide and cisplatin in murine tumor systems.

Based on that information, we began to evaluate the use of cyclophosphamide and VP-16-213 with or without cisplatin in a select group of poor-risk patients with both SQCLC and SMCLC. These patients were ineligible for our first-line protocols for these diseases which all included doxorubicin and cisplatin chemotherapy and surgical resection or thoracic irradiation if the disease was limited. The reasons for ineligibility were significant cardiac disease precluding the use of doxorubicin, significant renal disease precluding the use of cisplatin, significant pulmonary disease precluding the use of surgical resection or thoracic irradiation, or age > 73 years.

MATERIALS AND METHODS

Between May 30, 1978, and September 4, 1979, 48 poor-risk patients with measurable or evaluable SQCLC or SMCLC were entered in the study after a written informed consent was obtained. One patient was declared ineligible because no measurable or evaluable disease was present. Four patients with relatively normal cardiac, pulmonary, and renal functions were included because they were either unwilling or too ill to undergo the more extensive staging procedures required for the first-line protocols. All patients had to meet the following requirements: wbc count $\geq 4 \times 10^3/mm^3$; platelet count $\geq 130{,}000/mm^3$; total serum bilirubin < 2.0 mg/dl; Eastern Cooperative Oncology Group performance score (ECOG PS) ≥ 3; no uncontrolled infection; no prior combination chemotherapy; and no prior major surgery within 3 weeks.

Pretreatment evaluation consisted of a history

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and physical examination, a posteroanterior stereo chest roentgenogram, an ECG, a hematology and a chemistry group, and other tests as clinically necessary to assess the extent of disease and the patient's general medical condition. Between courses of chemotherapy, weekly wbc and platelet counts were obtained. Prior to subsequent treatments, a history and physical examination were performed and a chest roentgenogram and hematology and chemistry groups were obtained. Other pretreatment tests that had been positive for disease were done periodically, and always before a patient was declared to have achieved a complete regression (CR). Tissue confirmation of a CR was not mandatory.

Separate response criteria for measurable and evaluable disease were used. These criteria of response have been reported elsewhere (7). A partial regression (PR) of measurable disease was equivalent to a regression of evaluable disease. Both are considered less than a CR. Improvement or stability was not considered a regression.

In general, patients with serum creatinine values < 1.5 mg/dl were treated with CVP (cyclophosphamide, VP-16-213, and cisplatin) while patients with serum creatinine values ≥ 1.5 mg/dl were treated with CV (cyclophosphamide and VP-16-213).

In the CVP regimen, cyclophosphamide was given by rapid iv injection on Days 1–3 at a dose of 225 mg/m²/day (total dose per course, 775 mg/m²); VP-16-213 was given as a 30-minute infusion on Days 1–3 at a dose of 75 mg/m²/day (total dose per course, 225 mg/m²); and cisplatin was given on Day 1 at a dose of 60 mg/m² as a 4-hour infusion in 1600 ml of 5% dextrose in one-half normal saline with 25 g of mannitol added. In the CV regimen, the cyclophosphamide and VP-16-213 treatments were the same as in the CVP regimen. Initially, treatment was repeated at 3–4-week intervals but later could be repeated at 4–5-week intervals. If CNS metastases were present initially, this disease was irradiated immediately. After a minimum of six courses of chemotherapy, both prophylactic CNS and consolidative thoracic irradiation could be used in patients with SMCLC at the physician's discretion. Chemotherapy was discontinued after the completion of irradiation.

Of the 47 eligible patients, 31 had SMCLC and 16 had SQCLC. Of the 31 patients with SMCLC, seven were treated with CV (two with limited disease and five with extensive disease) and 24 were treated with CVP (11 with limited disease and 13 with extensive disease). Of the 16 patients with SQCLC, five were treated with CV (one with limited disease and four with extensive disease) and 11 were treated with CVP (four with limited disease and seven with extensive disease). About one third of the patients had an ECOG PS of 2–3 (47% of the patients with SQCLC, but only 29% of the patients with SMCLC). Almost 60% of the patients had significant cardiac disease and 13% had significant pulmonary disease. The patients' pretreatment characteristics are shown in table 1.

TABLE 1.—Pretreatment patient characteristics

Characteristic*	SQCLC		SMCLC	
	CV	CVP	CV	CVP
No. of patients entered	5	12	7	24
No. of evaluable patients	5	11	7	24
Stage				
Metastatic (extensive)	4	7	5	13
Locally unresectable (limited)	1	4	2	11
Serum creatinine (mg/dl)	1.7–2.0	0.7–1.3	1.2–2.1	0.6–1.4
Age (yrs)	62	62	61	65
Median range	60–73	51–78	49–73	46–75
ECOG PS				
0–1	2	6	3	19
2–3	3	5	4	5
Male:female	5:0	6:5	6:1	19:5
Measurable:evaluable	1:4	4:7	3:4	7:17
Cardiac disease	2	11	3	12
Pulmonary disease	0	3	0	3

*Unless specified otherwise, values = No. of patients.

RESULTS

Tumor Response

Twelve patients were treated with CV, five patients with SQCLC, and seven patients with SMCLC. No regressions were noted among the five patients with SQCLC; disease progression was seen in three patients after the first course and in two patients after the third course. Of the seven patients with SMCLC, three (43%) had tumor regressions (two CRs and one regression; one of two with limited disease and two of five with extensive disease), while four had tumor progression after one, three, three, and four courses of CV.

Thirty-five patients were treated with CVP, 11 patients with SQCLC, and 24 patients with SMCLC (11 with limited disease and 13 with extensive disease). Of the 11 patients with SQCLC, four (36%) had tumor regressions (one PR and three regressions). Six patients had tumor progression, five after one course of CVP and one after two courses. The final patient was stable for six courses after which he had the primary tumor resected when what was thought to be hepatic metastases by liver scan was revealed to be cirrhosis by liver biopsy. Nine (82%) of the 11 patients with limited SMCLC had tumor regressions, four CRs (36%) and five regressions. Of the two patients not obtaining a tumor regression, one died from drug toxicity at Day 12 and one had disease progression after one course. Ten (77%) of the 13 patients with extensive SMCLC had tumor regression, four CRs (31%), one PR, and five regressions. Of the three patients not obtaining a tumor regression, one had disease progression after one course and the other two withdrew from the study because of subjective toxicity while classified as having stable disease after one and three courses of CVP.

Durations of Regression

Durations of regression were calculated from Day 1 of treatment until (*a*) the first notation of tumor progression or (*b*) the date the patient was taken off the study for other reasons (eg, for the start of consolidative chest irradiation or prophylactic whole-brain irradiation). No patient with disease regression was taken off the study prior to the completion of at least six courses of chemotherapy and unless the known residual disease could be adequately irradiated (basically chest, supraclavicular fossae, and CNS). No patients with SQCLC responded favorably to CV. The three tumor regressions resulting from CV in patients with SMCLC lasted 213 (regression), 235 (CR), and 309 (CR) days.

The four regressions resulting from CVP in patients with SQCLC lasted 106+, 107, 329, and 432 days. Kaplan-Meier curves (8) of time to disease progression were derived for the 19 CVP-treated patients with SMCLC who had tumor regressions. The median duration of regression was 219 days for these 19 patients. The median regression duration was 219 days for the extensive-disease patients and was > 240 days (median not reached) for the limited-disease patients.

Survival

The patients with SQCLC survived poorly. The actual median survival times for the five patients treated with CV and for the 11 treated with CVP were 135 days (range, 12–506+) and 138 days (range, 29–523+), respectively. The four CVP-treated patients with tumor regressions are still alive at 106+, 138, 499+, and 523+ days.

The actual median survival time of the seven patients with SMCLC treated with CV was 269 days (range, 56–423). Median survival times for the 24 patients with SMCLC treated with CVP were estimated from a Kaplan-Meier curve (fig 1). For all 24 patients, the median survival time was 311 days (range, 12–507+) and ranged from 270 days for extensive-disease patients (range, 42–507+) to 371 days (range, 12–480+) for limited-disease patients. Using Gehan-Wilcoxon test statistics (9), the eight patients with CRs survived statistically longer ($P = 0.03$) than did the patients with less than a CR. Survival is estimated to be 75% at 420 days for the eight patients with CR versus a median of 270 days for the patients with less than a CR.

Sites of Disease Progression and Radiation Therapy (SMCLC)

Sites of initial disease progression are known in 18 patients. In the seven limited-disease patients (two receiving CV and five receiving CVP), all seven first had disease progression in the lung (one with liver and one with supraclavicular nodes also). In the 11 extensive-disease patients (five receiving CV and six receiving CVP) with known first sites of disease progression, the lung was involved in 45%, bone in 45%, liver in 27%, CNS in 18%, and lymph nodes in 9%. Nineteen of the 31 patients with SMCLC (61%) received radiation therapy to 26 sites in addition to their chemotherapy. No radiation therapy was given prior to the completion of six courses of chemotherapy unless (*a*) the CNS was involved at diagnosis or (*b*) there was disease progression. Sixteen sites (62%) were irradiated for tumor progression (seven to the CNS, four at di-

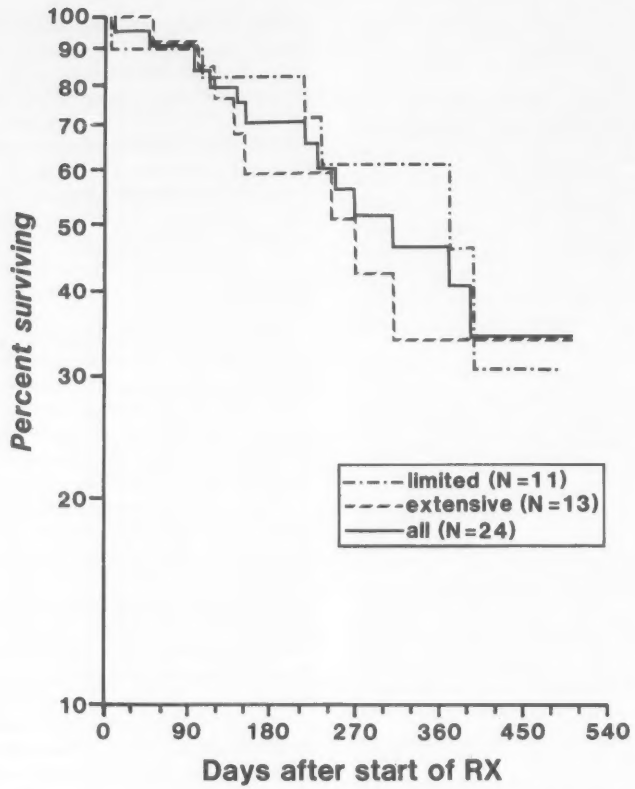

FIGURE 1.—Kaplan-Meier survival curves for the 24 patients with SMCLC treated with CVP.

agnosis; seven to the lung; one to the liver; and one to the bone). Prophylactic CNS irradiation was given to five patients. Thoracic irradiation to consolidate a less than CR was given to five patients.

Toxicity

Nausea and vomiting was infrequent and mild with CV but was essentially 100% and moderate to severe with CVP on Day 1 when cisplatin was administered. Elevations of serum creatinine > 1.2 mg/dl or baseline were noted in eight of 35 (23%) patients treated with CVP, but the elevation was no higher than 1.5 mg/dl in seven patients. The highest serum creatinine recorded was 2.3 mg/dl.

Hematologic toxicity after the first course of CV at protocol-prescribed doses was generally mild but became moderate to severe in later courses with upward dose adjustments of VP-16-213. Leukopenia was more significant and frequent than thrombocytopenia. Hematologic toxicity after the first course of CVP was generally mild to moderate but became moderate to severe in later courses with upward dose adjustments of VP-16-213. Leukopenia and thrombocytopenia seemed fairly equal in frequency and severity (table 2). Cisplatin augmented hematologic toxicity, particularly thrombocytopenia. In addition, hemoglobin levels generally decreased by 2–4 g/dl during treatment. No episodes of hemolysis were documented.

DISCUSSION

This study reports on the results of the use of cyclophosphamide and VP-16-213 with or without cisplatin in a select group of patients with SMCLC

TABLE 2.—Hematologic toxicity

	CV				CVP			
	SMCLC		SQCLC		SMCLC		SQCLC	
	1 course	All courses	1 course	All courses	1 course	All courses	1 course	All courses
Wbc count (cells × 10^3/mm^3) nadirs								
≥ 4	2*	1	0	0	12	4	1	1
3–3.9	3	1	3	2	4	5	0	0
2–2.9	0	0	0	1	5	8	1	0
1–1.9	0	3	0	0	1	5	5	6
< 1	1	1	0	0	1	1	0	0
Total evaluable	6	6	3	3	23	23	7	7
Platelet count (cells × 10^3/mm^3) nadirs								
≥ 130	6	4	2	1	16	8	2	1
100–129	0	2	1	2	4	7	1	1
50–99	0	0	0	0	3	6	0	1
< 50	0	0	0	0	0	2	0	0
Total evaluable	6	6	3	3	23	23	3	3

*Values = No. of patients.

or SQCLC. The major item of similarity among the patients was significant cardiac disease present in 60% of the patients (28 of 47 patients) treated. Significant cardiac disease implied a myocardial infarction within 3 months, congestive heart failure (in any state of compensation), cardiac arrhythmia requiring daily medication, or angina pectoris requiring daily nitroglycerin. In addition to the 28 patients with cardiac disease, another six patients (13%) had pulmonary disease too severe to permit surgical resection or limited-field irradiation of a tumor confined to the lung with or without involvement of regional nodes.

The CV regimen was disappointing. No regressions were noted in patients with SQCLC and only three regressions were noted in seven patients with SMCLC (although two were CRs). Our prior experience with CV in patients with limited-disease SMCLC resulted in nine regressions among 12 patients (one CR) after one course of therapy (4). The poorer regression rate noted here may be due to the more extensive disease and poorer performance score in these patients.

Cisplatin was originally added to CV because of its reported synergism with cyclophosphamide (6). Subsequently, we believe we have demonstrated a role for it in SQCLC (10), and Cavalli et al (11) and Dombernowsky et al (12) have reported some antitumor activity in SMCLC.

CVP seemed to be more effective than CV, although the study was not designed to test that comparison and the CVP patients generally had less extensive disease and better performance scores. Four regressions of good duration were noted among 11 patients with SQCLC. This regression rate (36%) is similar to that reported for cyclophosphamide, doxorubicin, and cisplatin (13); cyclophosphamide, doxorubicin, methotrexate, and procarbazine (14); and methotrexate, doxorubicin, cyclophosphamide, and CCNU (15), all of these regimens containing doxorubicin.

In SMCLC, CVP produced regressions in 79% of the patients (19 of 24 patients); 33% (eight of 24 patients) had "clinical" CRs. The median survival time of all 24 patients was 311 days (10.4 months) and ranged from 270 days (9 months) for patients with extensive disease to 371 days (12.2 months) for patients with limited disease. Comparing the 9-month median survival time for CVP in extensive-disease patients to other nondoxorubicin regimens or summaries, the following times are noted: 4.9 months for Edmonson et al (16), including 17% with limited disease (cyclophosphamide and CCNU); 8.5 months for Cohen et al (17) (high-dose cyclophosphamide, CCNU, and methotrexate); 5.7 months for Maurer et al (18) (cyclophosphamide, vincristine, and high-dose methotrexate); and 8.5 months for Bunn et al (19), a summary of 425 patients. Comparing the 12.2-month median survival time for CVP in limited-disease patients, the following times are noted: 9.3 months for Cohen et al (17) (high-dose cyclophosphamide, vincristine, and high-dose methotrexate); and 11.1 months for Bunn et al (19), a summary of 220 patients.

Recently, Sierocki et al (20) have reported on the use of VP-16-213 and cisplatin as an induction regimen in SMCLC followed by alternation with cyclophosphamide, doxorubicin, and vincristine. Prophy-

lactic whole-brain, but not consolidative thoracic, irradiation was used. The median survival time for all of Sierocki's 38 patients was 11 months and ranged from 9 months for extensive-disease patients to > 1 year for limited-disease patients.

In summary, CVP seemed to be more effective therapy than CV in patients with SQCLC or SMCLC although the difference could probably be explained, in part, by the more advanced disease and poorer performance score of the latter group. CVP was well-tolerated by patients with significant cardiac disease and resulted in median survival times, especially in patients with SMCLC, comparable to those noted in better-risk patients and to treatments with more morbidity (high-dose cyclophosphamide, CCNU, and methotrexate) or greater expense (cyclophosphamide, vincristine, and high-dose methotrexate). Further investigation of the role of cisplatin in the chemotherapeutic treatment of SMCLC and SQCLC should be undertaken.

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Phase I Trial of ICRF-187 by 48-Hour Continuous Infusion[1,2]

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SUMMARY

ICRF-187 is the D-enantiomer of the racemic antitumor agent ICRF-159 and was selected for clinical trials on the basis of aqueous solubility suitable for iv administration. Eighteen patients with refractory advanced solid tumors received ICRF-187 by a 48-hour continuous iv infusion in 5% Dextrose in Water, USP. The total dose ranged from 200 to 1000 mg/m²/48 hours. Courses were repeated at 22–28-day intervals. The major toxic effect was reversible granulocytopenia, with the nadir on Day 12 and the recovery by Day 22. At a dose of 1000 mg/m²/48 hours, the median nadir total wbc count was 1700/mm³ (range, 1300–2600), and the median nadir granulocyte count was 597/mm³ (range, 270–1300). Platelet count nadirs of < 100,000/mm³ occurred in only three of 16 courses at this level. Granulocyte toxicity was not cumulative and was less severe in repeated courses (median nadir, 1000/mm³ in courses two and three). Mild nausea, malaise, and three instances of alopecia were the only nonhematologic toxic effects encountered. Compared to other schedules, a continuous 48-hour infusion of ICRF-187 seems to have greater toxic effects for a given total dose, and this may predict greater biologic effect. A starting dose of 1000 mg/m² by a 48-hour continuous infusion is recommended for phase II trials.

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A variety of chelating agents have demonstrated inhibitory effects on cell growth, presumably by binding trace elements essential to enzyme function (1). The potent chelator EDTA has no antitumor activity because of high polarity that may prevent cellular uptake (2,3). Less polar derivatives were synthesized, resulting in the development of ICRF-159 (4). Although it is not a chelating agent in itself, ICRF-159 has shown a wide range of experimental antitumor activity. Activity was demonstrated in the L1210 leukemia system, with the optimal regimen being ip administration every 3 hours on Days 1, 5, and 9.[5,6] Synergism with radiation has been shown in the Sarcoma 180 system (5). Synergism with anthracycline antibiotics, 5-FU, and cisplatin has been observed in L1210 leukemia and in the Lewis lung tumor (6,7).

Antitumor activity in man is less apparent, although activity has been reported in human lung cancer (8), in colorectal carcinoma (9,10), and in Kaposi's sarcoma (11). No activity was noted in heavily pretreated patients with metastatic breast cancer (12) or melanoma (13). Measures which facilitate reliable delivery of ICRF-159 to the tumor may improve these results. The clinical pharmacology of ICRF-159 demonstrates rate-limiting gastrointestinal absorption following oral administration of radiolabeled compound (^{14}C-ICRF-159) (14). At a single dose of 3.0 g/m², urinary recovery of the

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[5]Wasserman TH, Friedman MA, Slavik M, et al. Clinical brochure: ICRF-159. Prepared by the National Cancer Institute, Bethesda, MD, Oct 1973.

[6]Clinical brochure: ICRF-187. Prepared by the National Cancer Institute, Bethesda, MD, June 1978.

label averaged only 23%. When this was given in three divided doses in 24 hours, recovery increased to 52%. The urinary activity appeared to be largely metabolite. The remaining radioactivity appeared mainly in feces and was largely ICRF-159, by chromatography. Predictable myelosuppression was not obtained at large single doses (up to 10.5 g/m²) but has been noted on all schedules when the drug is given in divided oral doses (15–17).

The erratic oral absorption of ICRF-159 led to efforts to develop a suitable parenteral formulation. ICRF-187 is the D-enantiomer of the racemate ICRF-159 and has aqueous solubility of 10 mg/ml, which permits continuous or intermittent iv administration in man (18).

The precise mechanism of action of ICRF-159 and ICRF-187 has not been established. ICRF-159 is active in late prophase and early metaphase (G_2/M phase) and inhibits DNA synthesis by an unknown mechanism, although it has been suggested that it could act as a bifunctional alkylator (19,20). However, prolonged contact of ICRF-159 with tumor cells may be necessary for maximum antitumor inhibition. Hellmann and Field (19) found that exposure of H.EP/2 cells to ICRF-159 for 1 hour had no effect on cell survival. A slow onset of drug "kill" was noted after prolonged exposure, with < 25% survival after 25 hours. This observation suggested that continuous infusions of ICRF-187 for periods > 24 hours might be more efficacious.

The major toxic effects of ICRF-187 in dogs included anorexia, nausea, emesis, diarrhea, lethargy, weight loss, hypothermia, and hematemesis. Leukopenia was common, but thrombocytopenia occurred less frequently. Elevations in SGOT were seen in every dog. SGPT and bilirubin were also often elevated. Fluctuations in BUN, alkaline phosphatase, and serum electrolytes were more sporadic and were not dose-related.

The toxic dose low for five daily doses in the dog was 625 mg/m², and the toxic dose low for a single dose was 10,000 mg/m². Thus, the drug was significantly more toxic with the regimen of daily doses for 5 days than with the single iv dose. We projected that this increase in toxic effects with increasing frequency of administration might extrapolate to man; thus, we started our phase I trial at a dose of 200 mg/m²/48 hours, which is one third of the highest nontoxic dose on the schedule of daily doses for 5 days.

MATERIALS AND METHODS

Patients with histologic evidence of advanced malignancy, who were not candidates for known effective regimens or protocol treatments and had recovered from toxic effects of prior treatment, were eligible for this study. Each patient had an Eastern Cooperative Oncology Group (ECOG) performance status of ≥ 3 and a life expectancy of at least 12 weeks. All patients who had received previous chemotherapy, immunotherapy, and/or radiotherapy had not received treatment for a minimum of 3 weeks and had no residual toxic effects. Patients were required to have adequate bone marrow function (wbc count ≥ 4000/mm³, absolute granulocyte count ≥ 2000/mm³, and platelet count ≥ 100,000/mm³), adequate liver function (bilirubin < 2 mg/100 ml and prothrombin time > 80% of normal), and adequate renal function (BUN ≤ 30 mg/100 ml, creatinine ≤ 1.5 mg/100 ml, and creatinine clearance > 60 ml/min). Informed consent indicating the investigational nature of the study was obtained from all patients according to institutional policy. Responses and toxic effects were defined by standard ECOG criteria (21).

Pretreatment evaluation included a complete history and physical examination, with documentation of metastatic disease and objective tumor measurement when possible. Laboratory studies included a cbc including differential, reticulocyte, and platelet counts, chemistry panel (BUN, creatinine, SGOT, alkaline phosphatase, total protein, albumin, total bilirubin, LDH, calcium, phosphorus, and uric acid), creatinine clearance, urinalysis, electrocardiogram, and chest roentgenogram. On Days 2 and 3 and weekly, cbc and chemistry panels were repeated. Repeat physical examinations with tumor measurements were performed at 3-week intervals.

ICRF-187 was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) in sterile vials containing 250 mg of lyophilized powder. Each vial was reconstituted with 25 ml of Sodium Chloride for Injection, USP, to a concentration of 10 mg/ml. One sixth of the calculated drug dose was administered in 250 ml of 5% Dextrose in Water, USP, over an 8-hour period by IVAC pump. This infusion was repeated six times for a total of 48 hours. The initial dose was 200 mg/m²/48 hours, with planned escalations to doses of 400, 666, and 1000 mg/m²/48 hours, according to the classic modified Fibonacci scheme. After communication with other investigators who were studying this agent, we modified our dose escalation scheme to omit the level of 400 mg/m²/48 hours and eventually reached a maximum dose of 1000 mg/m²/48 hours; at this dose, significant hematologic toxicity was encountered. Courses were repeated every 22–28 days, depending on adequate hematologic recovery.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	
Entered	18*
Evaluated	18
No. of courses	
Completed: 43	
Evaluated: 39	
Tumor type	
Breast	3
Lung	7
Ovarian	1
Colon	7
Prior therapy	
Chemotherapy	6
Radiation	1
Both	11
Neither	0
Performance status	
0–1	13
2–3	5

*1 patient escalated to next dose level.

RESULTS

Eighteen patients were entered in this phase I trial. A total of 43 courses of ICRF-187 were administered, and 39 of those courses were evaluable; four courses were unevaluable due to incomplete laboratory data supplied by community hospitals in terminal hospitalizations. Patient characteristics are shown in table 1. Almost all patients had received prior chemotherapy, usually with more than one agent, and the majority had received radiation therapy also.

Toxicity

Myelosuppression proved to be the dose-limiting toxic effect in this phase I trial. As shown in table 2, leukopenia was the most frequently observed hematologic effect of ICRF-187. Eleven of 15 courses at doses of 666 mg/m²/48 hours resulted in a wbc count nadir of ≤ 3000/mm³. The median absolute granulocyte count nadir at this dose was 1300/mm³. In three of the patients the absolute granulocyte count nadir occurred later than the wbc count nadir. At doses of 1000 mg/m²/48 hours, 21 of 22 courses resulted in wbc count nadirs of ≤ 3000/mm³, with the median wbc count nadir being 1750/mm³. The median absolute granulocyte count nadir was 597/mm³ at this dose. Again, in three patients the absolute granulocyte nadir occurred later than the wbc count nadir. One patient with a first-course wbc count nadir of 1600/mm³ received a second-course dose reduction of 20%, which produced a wbc count nadir of 3400/mm³. The third course was increased to the original dose, resulting in a wbc count nadir of 2100/mm³.

Cumulative leukopenia was not encountered. Slight increases in wbc count and absolute granulocyte count nadirs occurred in subsequent courses (table 3), despite dose escalations. The number of patients who received more than three courses is small, and the trend of the data must be validated by larger studies in the future.

Thrombocytopenia occurred less frequently than leukopenia. At doses of 666 mg/m²/48 hours, only three of 15 courses resulted in a platelet count nadir of ≤ 150,000/mm³. Three of 22 courses at doses of 1000 mg/m²/48 hours resulted in a platelet count nadir of ≤ 150,000/mm²; one patient had all three low platelet counts. Hematologic depression was generally maximal between Days 10 and 12 after start of ICRF-187, with recovery occurring within 7–10 days.

Acute nonhematologic toxic effects of ICRF-187 were generally mild and infrequent (table 4). Gastrointestinal toxicity, consisting of anorexia with occasional nausea and/or vomiting, did not seem to be dose-related, occurred within 24 hours after the infusion was stopped, and generally lasted 2–3 days. In three patients treated with doses of 1000 mg/m²/48 hours, alopecia developed 7–14 days after the infusion was stopped. No stomatitis or dermatitis was observed. No hepatic or renal toxicity was

TABLE 2.—Hematologic toxicity

Total dose (mg/m²/48 hrs)	No. of evaluable patients	No. of evaluable courses	Median wbc count nadir		Median platelet count nadir	
			Cells × 10^{-3}/mm³	Day	Cells × 10^{-3}/mm³	Day
200	3	3	6.8 (3.2–12)*	10	326 (257–525)	9
666	8	15	2.6 (1.7–4.4)	12	252 (95–488)	10
1000	8	22	1.75 (1.3–2.5)	12	198 (35–520)	10

*Values in parentheses = ranges.

TABLE 3.—Hematologic tolerance at doses of 1000 mg/m²/48 hrs

Course No.	No. of patients	Mean wbc count nadir (cells × 10^{-3}/mm³)	Mean absolute granulocyte count nadir (cells × 10^{-3}/mm³)
1	8	1.75	0.57
2	6*	2.14	0.98
3	4	2.25	1.04
4	2†	2.00	0.64
5	1‡	2.5	1.6
6	1§	2.3	0.023

*Patient with 20% dose reduction was excluded from evaluation.
†Both patients received a 20% dose escalation.
‡Maintained at 20% dose escalation from original dose.
§Received a 40% dose escalation from original dose.

TABLE 4.—Other toxic effects

Dose (mg/m²/48 hrs)	No. of evaluable patients	No. of patients with—			
		Nausea and vomiting	Anorexia	Malaise	Alopecia
200	3	1	2	1	0
666	8	4	2	3	0
1000	8	1	3	4	3

noted at any dose level. Extravasation of the drug in two patients caused no cutaneous reactions.

Response

While the major objectives of this study were to evaluate the dosage and toxicity of ICRF-187, observations were also made regarding tumor response. The standard ECOG criteria for response were observed. No objective tumor regression was noted in any patient. One patient with squamous cell carcinoma of the lung did continue to receive therapy for 24 weeks (six courses), with stable disease.

DISCUSSION

ICRF-187, the D-enantiomer of ICRF-159, was proposed for clinical trials on the basis of increased aqueous solubility permitting iv administration. Previous studies with ICRF-159 given orally in divided doses at a total dose range of 750 mg/m²/day to 1 g/m²/day × 3 showed myelosuppression affecting wbcs more than platelets, with a nadir count on Day 12 and recovery by Day 21. Leukopenia was severe at times, with thrombocytopenia mild and transient. Nausea and vomiting occurred in 30%–60% of the patients but was manageable with antiemetics. Alopecia was common, and occasional stomatitis, dermatitis, and a flu-like syndrome were reported (9,10,17).

For the 48-hour infusion of ICRF-187, myelosuppression proved to be the dose-limiting toxicity, affecting wbcs more than platelets; nadir counts and times to recovery were similar to those noted with ICRF-159. Cumulative myelosuppression did not occur; in fact, a modest tolerance seemed to develop in subsequent courses, with some patients receiving dose escalations. The nonhematologic toxic effects of ICRF-187 were likewise qualitatively similar to those of the parent racemate ICRF-159, although the symptoms of nausea, malaise, and vomiting were quite tolerable and amenable to control with conventional antiemetics. The most apparent difference is the relatively low total dose (666–1000 mg/m²) for a given level of hematologic toxicity as compared to ICRF-159, presumably due to the limited absorption of the parent compound. The spectrum of toxicity, as shown above, is virtually identical.

The purpose of this study was to define the dose of ICRF-187 that would produce acceptable toxicity in order to conduct disease-oriented trials. Based on our data, an appropriate starting dose for phase II studies would be 1000 mg/m²/48-hour infusion every 21–28 days. This dose may need to be escalated in subsequent courses as tolerated. The toxic effects of ICRF-187 in man are schedule-dependent,

with greater toxic effects at lower doses when given by 48-hour infusion than by intermittent bolus schedules (C. Haas, personal communication). Phase II studies may establish whether this results in increased antitumor efficacy. An investigation of the pharmacologic basis for this schedule-dependent toxicity is in progress.

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Phase II Evaluation of Methyl-GAG in Patients With Refractory Metastatic Breast Cancer[1,2]

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SUMMARY

Twenty-nine patients with metastatic breast cancer were treated with methyl-GAG at an initial starting dose of 500 mg/m² iv weekly and then escalated by 50 mg/m²/week to tolerance. There were two partial responders among the 26 evaluable patients. Gastrointestinal and cutaneous reactions were the limiting toxic effects of methyl-GAG. Myelosuppression was mild and infrequent.

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Since its first description in 1958 (1), methyl-GAG, a polyamine synthesis inhibitor, has been found to have substantial activity in early clinical trials, especially in acute myelogenous leukemia using a daily dose schedule (2–4). Unfortunately, toxic effects were prohibitive, including severe mucositis (especially laryngitis and esophagitis), vasculitis, profound anorexia with associated weight loss, hypoglycemia, and myelosuppression. Numerous attempts have been made to improve the therapeutic index of methyl-GAG; however, it was not until recently that renewed interest in methyl-GAG has led to the re-evaluation of the drug in a phase I–II study using a weekly iv administration with encouraging results (5). Significantly, there was a marked increase in the therapeutic index in that 74% of the treated patients exhibited no toxic effects. Based on this and hints of antitumor activity observed by the Eastern Cooperative Oncology Group in patients with advanced breast cancer treated with an intermittent im schedule of methyl-GAG (6), further exploration of the therapeutic potential of methyl-GAG in patients with advanced refractory metastatic breast cancer appears desirable.

PATIENTS AND METHODS

Twenty-nine patients with advanced metastatic breast cancer who had failed prior chemotherapy with doxorubicin, cyclophosphamide, 5-FU, methotrexate, and vinblastine; prior hormonal therapy with oophorectomy and tamoxifen; or both chemotherapy and hormonal therapy were entered in this study. Eligibility requirements include the presence of measurable disease and an International Union Against Cancer (UICC) performance score of ≤ 3. Patients were required to be off all prior hormonal therapy or chemotherapy for at least 4 weeks before entering the phase II study. Studies obtained prior to initiation of therapy consisted of medical history, physical examination with measurement of lesions, complete hematology survey including differential, wbc, and platelet counts, chemistry profile, chest roentgenogram, metastatic bone survey, and liver and bone scans when appropriate. All patients were reassessed every 3–4 weeks with repeated tumor measurements and selective x-ray studies. Written informed consent was obtained from all patients.

Initially, a starting dose of 500 mg/m² of methyl-GAG iv was administered weekly to all patients for the first 2 weeks. In the absence of toxic reactions following two treatments at the initial dose, a dose escalation of 50 mg/m²/week to tolerance was insti-

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tuted. The calculated dose was dissolved in 250 ml of 5% dextrose in water and was infused iv for a minimal period of 45 minutes.

RESULTS

Of the 29 patients entered in the study, 26 were evaluable for response. Reasons for inevaluability included death within 2 weeks from the start of therapy (two patients) or loss to followup after only one course of methyl-GAG (one). Pertinent patient characteristics are shown in table 1. All of these patients had received extensive prior chemotherapy. The median number of prior inductive regimens was four (range, two to nine regimens), with a median duration of prior treatment of 30 months (range, 12–71). Among this group of extensively pretreated patients, there were two partial responders. Both patients had soft tissue and osseous tumor involvement, and both responded following three weekly doses of methyl-GAG with a > 50% reduction in their soft tissue disease; subsequently, after additional weekly courses of therapy, there was evidence of blastic repair of their mixed lytic-blastic bone lesions. Responses lasted for 3 and 4 months. An additional eight patients achieved stability of their disease, including two patients who experienced a less-than-partial response and whose therapy was discontinued because of the presence of excessive toxic reactions. One of these two patients with diffused osseous involvement has had marked improvement in performance status as well as significant diminution in her serial blood carcinoembryonic antigen levels. Unfortunately, the patient developed corneal epithelial defects requiring discontinuation of additional therapy with methyl-GAG. Another patient has had significant symptomatic relief from her osseous metastases with < 50% reduction in her metastatic lung lesions. Therapy was discontinued in this patient because of the presence of anorexia, weight loss, and extensive skin rash with erythema nodosum. Fifteen patients had evidence of progressive disease following a minimum of three weekly doses of methyl-GAG.

Hematologic and nonhematologic toxic effects are shown in tables 2 and 3. Seventeen of the 26 patients had evidence of some toxic effects. Myelosuppression was seen in only five patients; granulocytopenia with an absolute granulocyte count < 1000/mm^3 occurred in 5% of the administered courses, and thrombocytopenia with a platelet count ≤ 100,000/mm^3 occurred in 19% of the administered courses. The most commonly encountered toxic effect was nausea and vomiting, which occurred in ten patients (38%). Five patients developed nodular bilateral thrombophlebitis affecting the lower extremities after 3–6 weekly doses of methyl-GAG, and in two of these patients it was associated with severe muscle pains and weakness. Erythema nodosum occurred in two patients. Generally, toxic reactions appeared to be cumulative, the frequency and severity increasing with the number of courses of methyl-GAG (7).

TABLE 1.—Patient characteristics

Characteristic*	Value
Total No. of evaluable patients	26
Age (yrs)	
Median	50
Range	32–74
Menopausal status	
Premenopausal	4 (16%)
Postmenopausal	21 (80%)
Unknown	1 (4%)
Disease-free interval (mos)	
Median	20
Range	0–246
UICC performance status	
≤ 2	17 (65%)
> 2	9 (35%)
No. of organ sites involved	
≤ 2	14 (54%)
> 2	12 (46%)
Dominant disease site	
Soft tissue	0
Osseous	12
Visceral	14
No. of prior treatments	
Median	4
Range	2–9
Duration of prior treatments (mos)	
Median	30
Range	12–71

*Unless specified otherwise, values = No. of patients.

TABLE 2.—Hematologic toxicity

	Lowest value (cells × 10^3/mm^3)
Total wbc count	
Median	3.4
Range	0.6–9.7
Absolute granulocyte count	
Median	2.6
Range	0.2–8.0
Platelet count	
Median	150
Range	45–439

TABLE 3.—Nonhematologic toxicity

Toxic effect	No. of patients (%)
Gastrointestinal	
Nausea	10 (38)
Vomiting	9 (34)
Stomatitis	2 (7)
Diarrhea	5 (19)
Anorexia	3 (11)
Others	
Skin rash	3 (11)
Erythema nodosum	2 (7)
Vasculitis (nodular bilateral thrombophlebitis)	5 (19)
Muscle pain (myositis)	2 (7)
Facial swelling	1 (3)
Hypotension	1 (3)
Rhinorrhea	1 (3)
Photophobia	1 (3)
Corneal epithelial defect	1 (3)

DISCUSSION

Previous experience with methyl-GAG suggests evidence of antitumor response in patients with carcinoma of the breast (6). More recently, Knight et al (5) reported a marked increase in the therapeutic index of iv methyl-GAG when given weekly in the treatment of refractory metastatic malignancies. Encouraging results were seen in patients with refractory colon, renal, and pancreatic cancers, but no complete or partial responses were observed among the 12 patients with refractory metastatic breast cancer. Although the types of toxicity described were similar, vasculitis and cutaneous toxic reactions were more frequently encountered in our patient population. The rather modest degree of antitumor activity coupled with significant toxicity observed in our patients suggests that methyl-GAG may be of little value in the treatment of patients with metastatic breast cancer who have failed extensive prior chemotherapy.

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Determination of Tissue Concentrations of Vinca Alkaloids by Radioimmunoassay[1,2]

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SUMMARY

Methodology for the determination of tissue concentrations of the vinca alkaloids has been developed with a specific radioimmunoassay (RIA). Data obtained with this RIA were compared with those obtained following direct administration of radiolabeled vinblastine. Distribution of radiolabeled vinblastine was also compared between control rats and those bearing the transplantable tumor Lymphoma 8. Vindesine concentrations in various rat tissues were determined via RIA over a 52-hr period.

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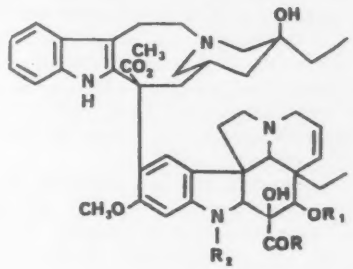
Vindesine (VDS) is a new synthetic derivative (fig 1) of vinblastine (VLB), for which phase I data have been reported (1–6) and phase II and III trials have now begun. Recently, the pharmacokinetic behavior of both VLB and VDS has been reported in man (7–11). Culp et al (12) reported the tissue distribution and disposition of radiolabeled VDS. Because of the importance of information that will increase our overall understanding and evaluation of these drugs, especially in looking forward to whole-body pharmacokinetic modeling, we now report the distribution and variation with time of VLB and VDS concentrations in the tissues of rats. Data on the distribution of radiolabeled VLB in tumor-bearing rats are also reported and compared with data obtained using an adaptation of the newly developed radioimmunoassay (RIA) for the vinca alkaloids in tissue samples (9,11,13).



	R	R_1	R_2
VLB	OCH_3	$COCH_3$	CH_3
VCR	OCH_3	$COCH_3$	CHO
VDS	NH_2	H	CH_3
DVLB	OCH_3	H	CH_3

FIGURE 1.—Structures for the vinca alkaloids.

MATERIALS AND METHODS

4-Acetyl-^{3}H-VLB was prepared as described earlier (10,14); it had a specific activity of 2.44 curies/millimol and was 90% isotopically pure. This VLB was diluted with unlabeled VLB and was administered to two groups of female Lewis rats at a dose of 1.4 mg/kg, found to be the most effective anti-Lymphoma 8 dose level (*see* table 1). One group of rats weighing 200–300 g was used to determine the distribution of the drug in normal rats. The second group of rats, weighing 110–150 g and bearing Lymphoma 8, was treated on Day 10 following subcu-

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taneous implantation of 10^6 viable spleen cells (routinely passed from animals bearing Lymphoma 8 that were 1–2 days preterminal). The animals were killed by cardiac puncture and exsanguination, and all tissues were stored at –20°C until analyzed. Portions of each organ were oxidized completely to tritium water (Packard Sample Oxidizer, model 305), and the sample was counted in 15 ml of Instagel (Packard).

Female Lewis rats weighing 200–250 g were given VDS iv at 0.25 mg total dose (1–1.25 mg/kg), and at each time point a single animal was killed by cardiac puncture and exsanguination. The tissues were then removed and weighed. Accurately weighed portions of these organs were then homogenized in 2:1 and 4:1 methanol:water solution in an all-glass tissue hand homogenizer, followed by centrifugation of the mixture at 7000 × *g* for 10 min. This procedure is simple, and because of the use of methanol, most of the proteins were denatured. Rarely did an emulsion form; when it did, it was easily broken by the addition of a few grains of solid NaCl and recentrifuging. The supernatant was then removed and the solvent was evaporated under reduced pressure. A measured volume of water, either 2.0 or 4.0 ml, was then added to the residue, and the mixture was agitated until solution was complete. This sample was either used directly in the RIA or stored at –20°C.

This procedure for the preparation of the various tissues worked very well. The only difficulty that arose was with the very dense tissues, such as muscle and skin, where considerable effort was required to assure adequate homogenization. To avoid photodecomposition of the vinca alkaloids, all procedures were carried out either in the dark or in a room with fluorescent light that had no ultraviolet light in its emission spectrum.

To perform the RIA, reagents were placed in 5-ml polyethylene tubes in this order: (*a*) 200 µl of glycine buffer (0.2 M glycine at pH 8.8, 0.25% crystalline human albumin [ICN, 4X recrystallized], 1% normal lamb serum [North American Biologicals], and 242 mg of Merthiolate/liter); (*b*) 100 µl of unknown or standard solution; (*c*) 100 µl of ^{3}H-VLB solution (Amersham, aromatic ring-labeled, 1.5 × 10^{-9} g/ml, specific activity of 8.2 curies/millimol, 95% isotopically pure); and (*d*) 100 µl of antiserum (diluted to 1:1000 with glycine buffer). The tubes were mixed gently, capped, and incubated 4 days at 4°C. To each tube was then added 500 µl of dextrancharcoal suspension (0.5% Norit A [Amend Drug and Chemical Co] and 0.25% dextran 70 [Pharmacia] in glycine buffer), and incubation was continued at room temperature for 30 min with occasional gentle shaking. The tubes were centrifuged at 1000 × *g* for 5 min, and the supernatant was decanted directly into scintillation vials. Instagel (10 ml) was added, and the vials were counted in a scintillation spectrophotometer.

The standard solutions contained unlabeled VDS (Eli Lilly Laboratories, supplied as 10 mg of sulfate salt together with 30 mg of mannitol in sealed vials) or VLB (as sulfate salt) in glycine buffer, with concentrations ranging from 0.2 to 100 × 10^{-9} g/ml. The following were also run: (*a*) 100 µl of nonimmune rabbit serum substituted for the antiserum, and (*b*) 100 µl of glycine buffer in place of standard or unknown. The supernatant was counted in both these controls.

Calculations were performed as follows:

$$\text{control 2–control 1} = \text{A}$$

$$\frac{\text{sample–control 1}}{\text{A}} \times 100 = \%\ \text{bound.}$$

The percent bound for the standard curve was plotted on logit paper, and the unknowns were then read from the linear portion of the graph.

The standard curve for the RIA as shown in figure 2 was linear over the concentration range of 2–60 ng/ml. As was found earlier (9,11), identical (within statistical limits) curves were obtained for VDS, vincristine (VCR), and VLB, with or without serum. For each set of samples run, a standard curve was simultaneously determined, and the unknown samples were analyzed according to that specific standard curve.

The only metabolic product known and identified for the vinca series is desacetylvinblastine (DVLB), and this drug had a standard curve identical to that of the parent VLB (11).

RESULTS

We determined that VLB was an effective drug in treating animals with Lymphoma 8, as shown in table 1. The maximum effective dose is estimated to be 1.4 mg/kg.

We dosed a series of rats with 4-acetyl-^{3}H-VLB to determine tissue concentrations and to compare control rats with preterminal animals carrying Lymphoma 8. At various times following an iv dose of 1.4 mg/kg of VLB (diluted to contain 0.500 microcuries of ^{3}H-VLB), the tissues of a control and a Lymphoma 8-bearing rat were examined for total radioactivity. These data were converted on the basis of original specific activity and are shown in table 2 as nanograms of VLB per gram of tissue. Relatively high concentrations of radioactivity were

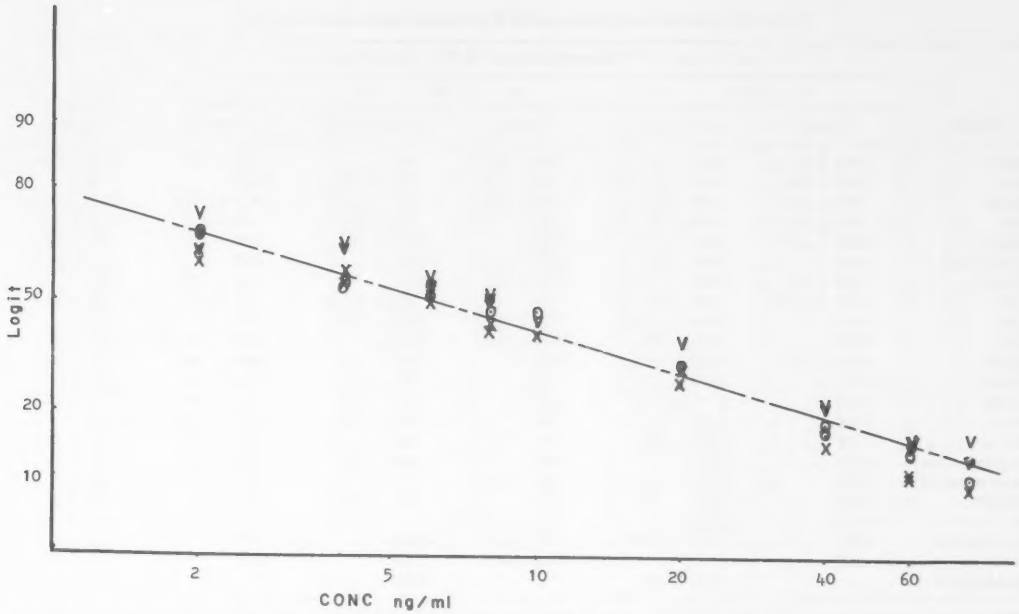

FIGURE 2.—RIA standard curve for VCR (○), VDS (X), and VLB (V). There was no statistical difference among the various points for these, and only one curve is shown. CONC = concentration.

present in tumor-bearing subcutaneous tissue and lymph nodes, especially in the 24-hr samples. This concentration was greater than that found in the adjacent skin and muscle. There was also a relatively high concentration in the marrow and a relatively low concentration in the brain. Overall, the concentrations found in the tissues of control animals roughly agreed with those found earlier using VLB that had been labeled via the Wilzbach procedure. This latter technique labels the aromatic ring protons by exchange with gaseous tritium but results in extremely low yields of labeled VLB and causes major problems in product separation and purity (15).

We next examined the application of the RIA to determination of tissue concentrations of the vinca alkaloids. From our prior experience and again in these studies, we found identical cross-reactivity of the antiserum with VLB, VCR, and VDS, inasmuch as the standard curves were superimposable. Since VLB was in plentiful supply, we first explored the recovery of this drug from various tissue homogenates. This was accomplished by adding a known amount of drug to a tissue sample, followed by homogenization in the methanol:water system described and an RIA run on the supernatant after centrifugation. We routinely recovered an average of 87% of the drug. Recovery of radioactive VLB,

TABLE 1.—Lymphoma 8 treated with VLB*

Dose (mg/kg)	Mean survival ± SD (days)	Comments
0	12.4 ± 1.5	Control
5.5	6.4 ± 1.1	Toxic
4.1	6.8 ± 0.8	Toxic
2.8	16.2 ± 1.5	
1.4	19.8 ± 1.9	1 indefinite survivor
0.7	16.2 ± 0.8	1 indefinite survivor

*Rats weighing 100 g were given 10^6 Lymphoma 8 cells subcutaneously on the right flank. On Day 3, doses of VLB were given iv to 5 rats at each level.

TABLE 2.—Distribution of 4-acetyl-^{3}H-VLB in rats following iv injection*

Tissue	Concentration of VLB in ng/g of tissue at—					
	2 hrs		24 hrs		3 days (control)	6 days (control)
	Control	Lymphoma 8	Control	Lymphoma 8		
Brain	69.6 ± 2.6	49.0 ± 0.9	395 ± 78	141	28.2 ± 10	28.4 ± 5.5
Bone	396 ± 236	673 ± 51	306 ± 164	302 ± 238	98.0	0
Muscle	679 ± 191	942 ± 129	360 ± 239	257 ± 239	56.4 ± 34.9	27.1 ± 18.2
Skin	321 ± 206	348 ± 96	147	137 ± 18	108 ± 14	19.3 ± 3.3
Thyroid	3394 ± 1773	2289 ± 544	1388	1021 ± 103	436 ± 18	126 ± 12
Lymph node	1525	3888 ± 351	1752	3125	698	171
Thymus	1450 ± 821	1324 ± 116	2702 ± 273	2176	718 ± 260	200
Heart	2183 ± 1528	1534 ± 187	337	383 ± 8	254 ± 32	88 ± 11
Lung	3441	4854 ± 1202	1710 ± 458	2332 ± 161	367 ± 45	146
Liver	3006	3996 ± 985	2345 ± 959	3131	738 ± 40	256 ± 9
Spleen	4042 ± 474	2588 ± 807	5321	382 ± 115	2099 ± 138	586 ± 28
Adrenal	13,656	6809 ± 127	774	334 ± 29	1430 ± 124	270 ± 4
Kidney	4167	2093 ± 750	452	640 ± 146	413 ± 40	158 ± 21
Ovary	3054	3223 ± 88	944	302 ± 145	1551 ± 154	1284
Uterus	1361	1009 ± 156	421	201	322 ± 26	107 ± 47
Small intestine	4832	1778 ± 90	600	445 ± 337	159 ± 24	50 ± 15
Large intestine	2089	1320 ± 124	491 ± 198	904 ± 9	287 ± 8	44 ± 9
Stomach	4859	2313 ± 301	869	501 ± 224	278 ± 76	80 ± 4
Fat	1917	2931 ± 127	437 ± 155		134 ± 87	0
Bone marrow	9570	7535 ± 1496	844 ± 171	1263	549	0
Blood	239		82.1 ± 0.9	28.9	16.9	0
Lymphoma 8		823 ± 164		1294		

*All animals were dosed at 1.4 mg/kg of VLB. Values = mean ± SD for 2 animals per time point. Where no SD is listed, only 1 rat was used.

added to the tissues in a similar manner, averaged 84%. All data displayed are uncorrected for recovery.

We dosed three adult female 200-g Lewis rats with VLB at 0.5 mg/kg and killed them at various times by cardiac exsanguination. The liver, spleen, and kidneys were isolated from each animal and stored at –20°C. Using the above RIA technique,

TABLE 3.—Distribution of VLB in rats as measured by RIA, ^{3}H-VLB, and 4-acetyl-^{3}H-VLB

Method*	Rat No.†	Concentration of VLB in ng/g of tissue‡			
		Spleen	Kidney	Liver	Serum
		2 hrs after dose			
RIA	1	3391 ± 831	1225 ± 71	712 ± 72	27.3 ± 4.8
	2	1160 ± 181	687 ± 187	494 ± 110	17.1 ± 1.4
	3	1794 ± 277	675 ± 66	417 ± 48	
	Average	2167 ± 1237	775 ± 259	542 ± 141	
^{3}H-VLB		1623	1151	1808	
4-acetyl-^{3}H-VLB		1444	1488	1074	
		24 hrs after dose			
RIA	1	260 ± 41	58 ± 9	20.8 ± 7.0	3.4 ± 1.9
	2	231 ± 77	63 ± 11	12.9 ± 2.6	3.6 ± 1.6
	3	362 ± 79	73 ± 14	27.6 ± 5.7	
	Average	266 ± 75	61 ± 11	19.8 ± 7.7	
^{3}H-VLB		577	240	423	
4-acetyl-^{3}H-VLB		1900	161	838	

*RIA values are uncorrected for amount of recovery (87%). ^{3}H-VLB data were calculated from (15) on the basis of 0.5 mg/kg of VLB. 4-Acetyl-^{3}H-VLB values are from table 2, corrected to 0.5 mg/kg of VLB.

†Rats received a dose of 0.5 mg/kg of VLB iv.

‡Values = mean ± SD.

TABLE 4.—Distribution of VDS in rats as measured by RIA

Tissue	Concentration of VDS in ng/g of tissue at—				
	2 hrs	7 hrs	12 hrs	24 hrs	52 hrs
Brain	7.4 ± 3.7*	12.5 ± 2.2	9.6 ± 4.1	4.6 ± 0.3	5.9 ± 1.9
Eye	7.5 ± 1.0	26 ± 8.5	26 ± 4.3	10.6 ± 2.2	14.3 ± 0.8
Thyroid	62 ± 14	713 ± 11	443 ± 53	348 ± 81	392 ± 44
Lung	217 ± 15	642 ± 176	229 ± 35	55 ± 7	58 ± 6
Heart	86 ± 2	318 ± 61	260 ± 90	47 ± 11	23 ± 7
Spleen	1070 ± 280	1170 ± 110	1570 ± 440	856 ± 125	1687 ± 168
Liver	409 ± 104	541 ± 138	142 ± 37	71 ± 8	73 ± 30
Kidney	332 ± 21	660 ± 79	302 ± 16	200 ± 53	111 ± 4
Adrenal	45	1900 ± 495	1060 ± 11	683 ± 11	512 ± 157
Small intestine	824 ± 156	963 ± 18	358 ± 127	136 ± 82	152 ± 18
Large intestine	243 ± 22	489 ± 203	479 ± 39	210 ± 44	150 ± 43
Skeletal muscle	82 ± 68	94 ± 26	63 ± 28	40 ± 17	18 ± 1.7
Skin	0	79 ± 28	136 ± 58	32 ± 5	18 ± 4.5
Bone marrow	21 ± 0	628 ± 40	870 ± 64	266 ± 53	151 ± 40
Fat	0	14.4 ± 5.7	11.8 ± 2.4	6.1 ± 2.0	13.5 ± 1.8
Ovaries and uterus	261 ± 46	134 ± 2	109 ± 3	111 ± 18	48 ± 9
Serum	50 ± 11	27.5	8.3 ± 0.1	8.3 ± 0.8	2.7 ± 0.5
Animal weight (g)	254	255	251	259	191
Mg/kg of VDS	0.98	0.98	1.00	0.97	1.00

*Values = mean ± SD for 2 animals per time point. Where no SD is listed, only 1 rat was used.

we measured the concentration of VLB in each sample.

Liver, spleen, and kidneys were used since of all organs in the rat, they had been found to have the highest levels of radiolabeled VLB (15). The VLB concentrations found are displayed in table 3, together with prior data obtained using two forms of radioactive VLB: 4-acetyl-^{3}H-VLB (present study) and VLB labeled in the aromatic ring (^{3}H-VLB) (15). Of great interest was the observation that at 2 hrs after dosing, the spleen and kidney had comparable VLB levels whether measured by RIA or ^{3}H-VLB, while the liver had much more radioactive material than could be accounted for by the RIA. This same pattern of high radioactivity in the liver persisted at 24 hrs, although at 24 hrs the spleen and kidney also had higher values as measured by ^{3}H-VLB than by RIA. We have no explanation for the variation in the 2-hr spleen and kidney RIA data for animal 1 versus animals 2 and 3.

We next determined the distribution of VDS in rats. Each animal was dosed with 1.0 mg/kg of VDS iv, and at various times, the animals were exsanguinated by cardiac puncture. Tissues were isolated, homogenized, and analyzed for VDS by RIA (table 4). We noticed that except for the serum and the reproductive system, the values all rise from 2 to 7 hrs and then fall at 12, 24, and 52 hrs. This pattern is reminiscent of that seen for VCR (16), although the rise in VDS at 7 hrs is more than that noticed for VCR.

DISCUSSION

We have developed a new and reliable technique for the determination of vinca alkaloid concentrations in mammalian tissues, by adaptation of a radioimmunoassay in which standard curves are nearly superimposable for VLB, VCR, DVLB, and VDS (structures shown in fig 1) (9). The antiserum is directed to the velbanamine portion of the molecule, such that alterations there could alter binding to the antiserum, while changes in the vindoline portion should not alter binding (13). The compounds in figure 1 show no loss of sensitivity to the antiserum, since all changes of structure lie only in the velbanamine portion of the molecule. One might anticipate that further changes similar to these, as perhaps appear in early metabolic conversion products, would also retain cross-reactivity. However, the full metabolic fate of VDS is unknown. VLB, on the other hand, is known to be altered to DVLB as a major metabolite. This compound has perhaps more biologic activity than the parent VLB (11). The remaining metabolic products of VLB were found to be noncytotoxic (11).

We suspect that the differences seen in the ^{3}H-VLB and RIA data may arise from two sources. When ^{3}H-VLB was first prepared, it was via the Wilzbach technique, which is known to generate many by-products, especially dihydrovinblastine (15). Although in this original work the authors recrystallized the ^{3}H-VLB to constant specific activ-

ity, the physical properties of VLB and dihydrovinblastine are very close, so that some dihydro derivatives may still have been present. Secondly, and we feel more importantly, while VLB may be quite drastically metabolically altered, the (Wilzbach) ^{3}H-VLB could still retain its tritium label (located in the aromatic ring) and remain sequestered in the various tissues as amphoteric or other products. These metabolic alterations (except for conversion to DVLB) are probably of sufficient magnitude to cause loss of cytotoxicity as well as loss of reactivity to the antiserum. With 4-acetyl-^{3}H-VLB, the ready loss of ^{3}H-acetic acid (known to occur [11]) to DVLB would separate the label from the drug. So, at later times, the distribution of radioactivity reflects not only unchanged ^{3}H-VLB but also the presence of ^{3}H-acetic acid and its metabolic products. For this reason and because in humans the non-VLB and non-DVLB materials had no cytotoxic activity in vitro (11), we suspect that the RIA data are more representative of biologically active vinca alkaloids in tissues. We concede the possibility that a noncytotoxic compound could be the causative agent in neuropathic changes, but we think it unlikely.

VLB has a definite antitumor effect on Lymphoma 8 (table 1), and appreciable concentrations of radioactivity were found in the tumor tissue and the lymph nodes of Lymphoma 8-bearing animals, suggesting that there is good uptake of VLB by the tumor. This correlation of antitumor effect and tissue uptake at the maximally effective dose of 1.4 mg/kg was most interesting, particularly at 2 hrs when little, if any, metabolic conversion had probably taken place. How representative the values are at 24 hrs we do not know, since we have no clear appreciation of drug:metabolite ratios in the tissues of these animals. Assuming the data to represent drug levels, we found that VLB concentrations in tumor tissue were of the same order of magnitude as found in other tissues. From this information, we cannot relate the cytotoxic effect of VLB to selectively high concentrations of the drug in tumor tissue. We can only speculate that other parameters, such as a high growth fraction in the tumor cell population may be involved, but since the growth fraction was not determined in this study, we cannot say for sure.

Of particular interest was the observation that, similar to VLB and VCR (15,16), very little VDS penetrated the CNS.

When we compared our present RIA data for VDS tissue concentrations with the values found by Culp et al (12) who used ^{3}H-VDS, we found that in general the data at 2 hrs were comparable for brain, lung, heart, spleen, kidney, and serum when adjusted for dose-level differences. Culp's ^{3}H-VDS values in muscle, skin, and fat, however, were higher than our VDS values determined by RIA. This may represent the concentration of tritium impurities, though it may just as readily be exchange of tritium out of the labeled molecule or that our extraction process needs refinement for these tissues, since they are very hard to homogenize. The high concentration of VDS measured in the adrenal gland using the RIA technique varies from the ^{3}H-VDS data, though we had earlier found very high concentrations of both ^{3}H-VLB and ^{3}H-VCR in adrenal tissue (16). One discrepancy in our data for VLB indicates concentrations of the drug in ovary and adrenal tissue that are lower at 24 hrs than at 3 days in normal rats, and we have no explanation for this.

We expect that these present data, together with the kinetic behavior of VDS in the serum of humans (7–9) will provide the basis for more complete pharmacokinetic modeling of this drug. This in turn should lead to better assessment of drug schedules and provide a rational basis for improved VDS administration to humans.

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Systemic Chemotherapy for a Primary Germ Cell Tumor of the Brain: A Pharmacokinetic Study[1,2]

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SUMMARY

Systemic administration of most chemotherapeutic agents has been assumed to be ineffective in the treatment of primary and metastatic brain tumors because these agents fail to cross the intact blood-brain barrier. However, agents which fail to penetrate the intact blood-brain barrier may penetrate it under conditions which include the presence of tumor in the central nervous system (CNS) and prior CNS irradiation. This paper reports the results of pharmacokinetic studies of bleomycin, cisplatin, and vinblastine in the CNS of a patient with a primary germ cell tumor of the brain who had received prior radiotherapy. Significant concentrations of bleomycin and cisplatin, but not of vinblastine, were reached in the cerebrospinal fluid (CSF) of the patient following iv administration. The area under the bleomycin CSF concentration times time curve was 25% of the area under the bleomycin plasma concentration times time curve. The areas under two cisplatin CSF curves were 50% and 155% of the areas under the corresponding free cisplatin plasma curves. Moreover, an objective response of the tumor to the chemotherapy was documented. This study provides evidence that, under certain circumstances, significant concentrations of cisplatin and bleomycin may be obtained in human CSF following systemic administration and that it may be possible to treat primary or metastatic CNS tumors with agents effective against systemic tumor of the same histologic type.

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Chemotherapy has played a limited role in the treatment of primary and metastatic brain tumors. Intrathecal administration of chemotherapeutic agents has been restricted to a small number of drugs and is generally effective only in the treatment of meningeal involvement with tumor. Systemic administration of most chemotherapeutic agents has been assumed to be ineffective because these agents fail to cross the intact blood-brain barrier. However, chemotherapeutic agents which fail to penetrate the intact blood-brain barrier may penetrate it under certain conditions. First, there is evidence to indicate that the blood-brain barrier may not be intact when either metastatic or primary tumor is present. An abnormally fenestrated and discontinuous endothelium has been described in both virally induced mammalian brain tumor models (1) and in human primary (2) and metastatic (3) brain tumors. Moreover, the passage of molecules excluded by the normal brain has been demonstrated in animal brain tumor models (4). Second,

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high-dose central nervous system (CNS) irradiation in mice has been shown to alter the blood-brain barrier sufficiently to allow previously impermeable drugs to penetrate (5). These observations suggest that in certain circumstances the systemic administration of at least some chemotherapeutic agents might be much more effective in the therapy for CNS tumors than had previously been thought.

This paper reports the results of pharmacokinetic studies of three chemotherapeutic agents administered systemically to a patient with a primary germ cell tumor of the brain. These studies consisted of the serial examination of both peripheral blood levels and cerebrospinal fluid (CSF) levels of each drug.

CASE REPORT

The patient is a young man who was first seen at our Medical Center at age 11 with complaints of decreased vision and headaches of 3 months' duration. His parents noted that since 5 years of age the patient had exhibited unusually avid thirst and frequent urination. Physical examination revealed a small boy who showed no signs of secondary sexual development. The optic discs showed temporal pallor bilaterally, and visual field examination revealed bitemporal field defects, most pronounced on the left side. The remainder of the neurologic examination was normal. Following a complete evaluation, during which panhypopituitarism was confirmed and an intrasellar tumor was delineated by arteriography, a right frontal craniotomy was performed. A large intrasellar tumor was partially resected to decompress the optic chiasm. Histologic examination of the tumor revealed a dysgerminoma. Following craniotomy the patient was treated with 5000 rads to the right and left pituitary region in 25 fractions through parallel opposed fields. The patient had nearly normal vision following therapy and was asymptomatic for 7 years until he again noted progressive visual difficulties. Bilateral optic atrophy and visual field defects were discovered during examination. Computerized axial tomography (CAT) of the brain showed a suprasellar tumor which had not been present 1 year previously. An additional 4000 rads of radiotherapy were given to the suprasellar tumor in 20 fractions but no improvement in the patient's vision resulted.

One year later further deterioration in the patient's vision and progressive memory loss were evident. CAT of the brain delineated a more extensive tumor than had been seen the previous year.

Because the patient had an inoperable tumor and had already received 9000 rads to the area of the tumor, we elected to attempt treatment of the tumor with a systemically administered combination of chemotherapeutic agents which has had great success in the treatment of germ cell tumors of testicular origin: cisplatin, bleomycin, and vinblastine (6). With informed consent, a craniotomy was first performed in order to attempt decompression of the optic chiasm and to place a Rickham reservoir in the right frontal ventricle. The Rickham reservoir was inserted in order to facilitate collection of CSF for pharmacokinetic studies of the chemotherapeutic agents. Decompression was found to be technically unfeasible and no tumor was removed during this procedure. Prior to chemotherapy the patient had elevation of the beta subunit of human chorionic gonadotropin (HCG) in both CSF (1150 mIU/ml) and serum (1200 mIU/ml).

After two courses of therapy the patient's memory improved significantly. There was minimal improvement in his visual acuity. A repeat CAT study revealed 75% shrinkage of the tumor. Moreover, the HCG levels in both CSF and serum declined to 250 and 5 mIU/ml, respectively. The use of CSF levels of HCG in the evaluation of patients with both primary and metastatic CNS germ cell tumors has been previously described (7–9). The patient remained in remission while receiving therapy for 7 months.

METHODS

Pharmacokinetic studies on peripheral blood and CSF were performed following the iv administration of bleomycin, vinblastine, and cisplatin. Bleomycin pharmacokinetic studies were performed on two occasions following the administration of 30 units of bleomycin by iv bolus. Vinblastine pharmacokinetic studies were performed on three occasions following the administration of vinblastine by iv bolus. On two occasions the dose of vinblastine administered was 0.15 mg/kg/day × 2 days (total dose, 12.8 mg/day). On the third occasion the dose of vinblastine was decreased to 0.10 mg/kg/day × 2 days (total dose, 9.6 mg/day). Cisplatin pharmacokinetic studies were performed on three occasions following 20-minute iv infusions of cisplatin. On the first occasion the dose of cisplatin administered was 20 mg/m²/day × 5 days (total dose, 35 mg/day). On the second occasion the dose of cisplatin was 50 mg/m²/day × 2 days (total dose, 75 mg/day). On the third occasion a dose of 37.5 mg/m²/day × 2 days was given (total dose, 50 mg/day).

Blood and CSF levels were obtained just prior to the administration of each chemotherapeutic agent and at various times from 2 minutes to 24 hours following the administration of each agent. The serial blood samples were obtained from a heparin

lock placed in the arm contralateral to the site of drug administration. The serial CSF samples were obtained by repeated punctures of the Ommaya reservoir under sterile conditions.

Bleomycin and vinblastine levels in the serum, plasma, and CSF were determined by radioimmunoassays using previously described techniques (10,11). The limit of sensitivity of the bleomycin assay is 5 microunits/ml while that of the vinblastine assay is 2 ng/ml. Total and free platinum concentrations were measured in the plasma and CSF. Centrifugation in Centriflo Membrane Cones (Amicon CF50A, Lexington, MA) was used to obtain an ultrafiltrate of plasma and CSF containing free (nonprotein-bound) platinum. The platinum in the biologic fluids was first oxidized with nitric acid and then complexed with ammonium pyrrolidine dithiocarbamate. The complex was extracted and concentrated in methyl isobutyl ketone, and platinum concentrations were measured by flameless atomic absorption spectrometry (12). Platinum concentrations were converted to cisplatin-equivalent concentrations by multiplying the nanograms of platinum per milliliter by 1.54 to determine the nanograms of cisplatin equivalents per milliliter.

The plasma or serum and the CSF concentration times time (C × t) data obtained for each drug were fitted by computer to single exponential, multi-exponential, or power equations. The elimination half-life of each drug from the blood and from the CSF was determined from the terminal slope of the computer-fitted C × t curves. Where appropriate, the area under the C × t curves was calculated by integration of the equations from Time 0 to infinity.

RESULTS

Bleomycin levels obtained in the plasma and the CSF following iv bolus administration of 30 units of bleomycin on one occasion are shown in figure 1. The earliest plasma level of bleomycin was measured at 15 minutes and was 3.45×10^3 microunits/ml. Bleomycin was rapidly cleared from the plasma in a biexponential fashion, with an α-half-life of 0.25 hours and a β-half-life of 1.32 hours. The volume of distribution of bleomycin was 11.97 liters (26.6% of body weight). These data are consistent with previously reported data (13). The peak CSF level of bleomycin, 2.24×10^2 microunits/ml, was obtained 2 hours following the administration of bleomycin. The peak CSF level was 40% of the simultaneously obtained plasma level. Bleomycin was cleared from the CSF more slowly than from the plasma, with an elimination half-life of 128 minutes. The area under the CSF C × t curve was 25% of the area under the plasma C × t curve. Similar data were

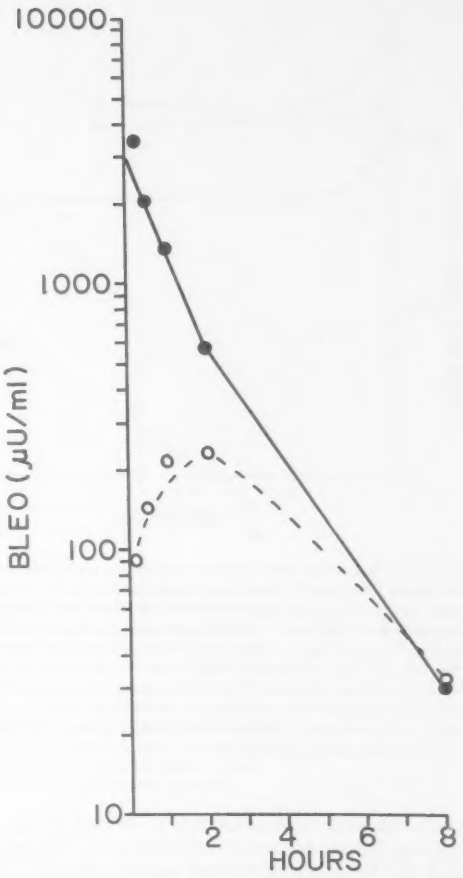

FIGURE 1.—Bleomycin levels obtained in the plasma (●) and CSF (○) after an iv bolus of 30 units of bleomycin. Symbols = actual data points. Solid line = computed curve drawn by fitting the actual plasma level data to a biexponential equation. Interrupted line = computed curve drawn by fitting the actual CSF level data to the sum of a power curve and a single exponential curve.

obtained following iv bolus administration of 30 units of bleomycin on a second occasion.

Total and free cisplatin equivalent levels obtained in the plasma following termination of 20-minute iv infusions of cisplatin are shown in figure 2. The peak plasma levels of both total and free cisplatin equivalents were proportional to the dose of cisplatin administered. Total cisplatin was cleared slowly from the plasma, with a β-half-life of approximately 38 hours. Free cisplatin equivalent levels were measured on ultrafiltrates of the plasma samples col-

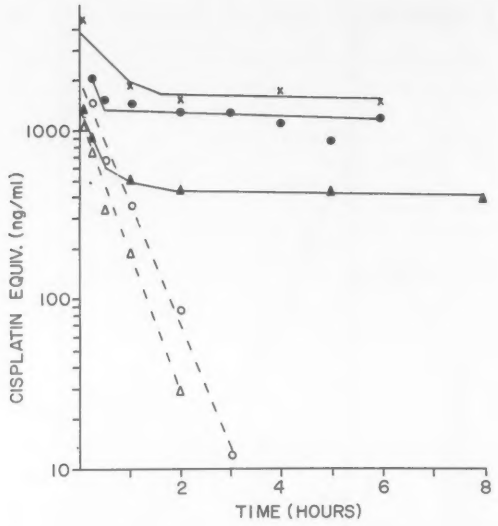

FIGURE 2.—Total and free cisplatin equivalent levels obtained in plasma following iv infusions of cisplatin. Symbols = actual data points: x = total cisplatin equivalents following 75 mg of cisplatin; ● = total and ○ = free cisplatin equivalents following 50 mg of cisplatin; ▲ = total and △ = free cisplatin equivalents following 35 mg of cisplatin. Solid lines = computed curves drawn by fitting the actual total plasma level data to equations describing the sum of 2 exponentials. Interrupted lines = computed curves drawn by fitting the actual free plasma level data to single exponential equations.

lected following administration of 35 and 50 mg of cisplatin. Due to technical difficulties, ultrafiltrates could not be obtained on the plasma samples collected following administration of 75 mg of cisplatin. Peak free plasma cisplatin equivalent levels were 70%–80% of the peak total plasma cisplatin equivalent levels. Free plasma cisplatin was rapidly cleared, with a half-life of 23.7 (± 1.6 SD) minutes. The short half-life of free plasma cisplatin is consistent with previously reported data (14).

Total and free cisplatin equivalent levels were determined on the CSF samples collected following iv administration of 50 mg of cisplatin. No substantial differences between the total and free levels were found. Figure 3 shows the cisplatin equivalent levels in the CSF and the free cisplatin equivalent levels in the plasma following termination of iv infusions of 35 and 50 mg of cisplatin. Peak cisplatin equivalent levels in the CSF were reached approximately 30 minutes following cisplatin administration. The peak levels in the CSF were 25% of the peak free cisplatin equivalent levels in the plasma. For cisplatin equivalent CSF, the areas under the C × t curves were 50% and 155% of the areas under the corresponding free cisplatin equivalent plasma C × t curves. The CSF levels of cisplatin equivalents following administration of 35, 50, and 75 mg of cisplatin are also compared in figure 3. Similar to the disappearance of free plasma cisplatin, the disappearance of cisplatin from the CSF occurs at the same rate at each dose level with a half-life of 1.44 (± 0.12 SD) hours.

The vinblastine levels obtained in the patient's serum following iv bolus administration of 12.8 mg of vinblastine on one occasion are shown in figure 4. Vinblastine was cleared from the serum, with an α-half-life of 2 minutes and a β-half-life of 3 hours. Similar data were obtained following iv bolus administration of 12.8 mg of vinblastine on a second occasion and 9.6 mg of vinblastine on a third occasion. The mean α-half-life was 2.3 (± 0.57 SD) minutes while the mean β-half-life was 3.8 (± 0.76 SD) hours. No significant levels of vinblastine (levels > 2 ng/ml) could be detected in the CSF at time points ranging from 2 minutes to 24 hours following vinblastine administration.

DISCUSSION

There are few pharmacokinetic studies of bleomycin, vinblastine, or cisplatin which include examination of CNS levels of the drugs. Most studies designed to determine the presence of bleomycin or vinblastine in the CNS of experimental animals following systemic administration have examined homogenized whole-brain tissue rather than CSF. Such studies in normal rabbits and mice failed to show significant levels of bleomycin, assayed microbiologically, in brain tissue, following systemic administration (15,16). Radioactivity was not detected in the brain of KHJJ tumor-bearing mice from 2 to 48 hours following the systemic administration of ^{131}I bleomycin (17). However, in 49 of 53 patients with brain tumors who received an iv bolus of 1 mg/kg of bleomycin 2 hours prior to craniotomy, bleomycin was detected by microbiologic assay in the surgically resected tumor tissue. Significant levels of bleomycin were not found in normal brain tissue which was also removed in 14 cases nor in eight CSF samples collected during surgery (18). Tritium-labeled vinblastine was not found in significant concentrations in normal rat brain following its systemic administration (19).

Pharmacokinetic studies of cisplatin in the CSF of monkeys equipped with Ommaya reservoirs following iv administration of the drug have recently been reported (20). A maximum concentration of

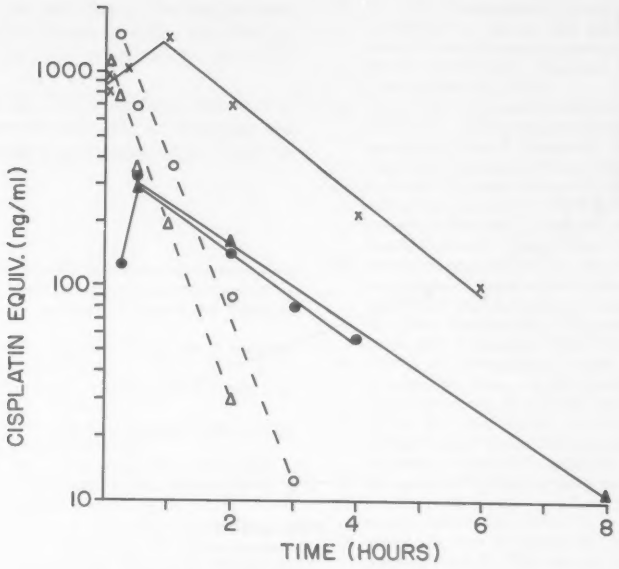

FIGURE 3.—Free cisplatin equivalent levels obtained in the plasma and cisplatin equivalent levels obtained in the CSF following iv infusions of cisplatin. Symbols = actual data points: x = cisplatin equivalent levels in CSF following 75 mg of cisplatin; ● = cisplatin equivalent levels in CSF following 50 mg of cisplatin; ▲ = cisplatin equivalent levels in CSF following 35 mg of cisplatin; ○ = free cisplatin equivalent levels in plasma following 50 mg of cisplatin; △ = free cisplatin equivalent levels in plasma following 35 mg of cisplatin. Solid lines = computed curves drawn by fitting the actual CSF level data to composites of 2 single exponential equations. Interrupted lines = computed curves drawn by fitting the actual free plasma level data to single exponential equations.

platinum in the CSF was found 30–40 minutes following iv injection of the drug. The ratio of CSF platinum levels to plasma platinum levels did not exceed 0.04 at any time. Platinum was cleared from the CSF rapidly, with a half-life of approximately 60 minutes.

The studies reported here are the first pharmacokinetic studies of vinblastine, bleomycin, and cisplatin in the CSF of man. These studies provide evidence that under certain circumstances significant concentrations of bleomycin and cisplatin can be obtained in human CSF following iv administration. The peak levels of bleomycin and cisplatin obtained in the CSF are less than the peak levels obtained in plasma. However, analysis of the areas under the C × t curves shows that the CSF had an exposure to cisplatin equivalent to that of the plasma. The exposure of the CSF to bleomycin was approximately 25% of the exposure of the plasma to bleomycin. In contrast, significant concentrations of vinblastine were not obtained in the CSF from 2 minutes to 24 hours following iv administration.

On the basis of the available data, it was predicted that vinblastine would fail to cross the blood-brain barrier and to reach significant concentrations in the CSF. It was expected that bleomycin, while possibly crossing the blood-brain barrier to reach the tumor, would not reach the high CSF concentrations observed in this study. Also, a much lower concentration of cisplatin in the CSF was predicted. The enhanced permeability of the patient's blood-brain barrier to these drugs may be due to the large volume of tumor present, the extensive prior radiotherapy, or a combination of these factors.

Vinblastine was excluded from the CSF of the patient, as expected from the limited animal data available. However, our data do not exclude the possibility that vinblastine may be bound to the tumor or to normal brain tissue. Indeed, one study which examined homogenized mammalian brain tis-

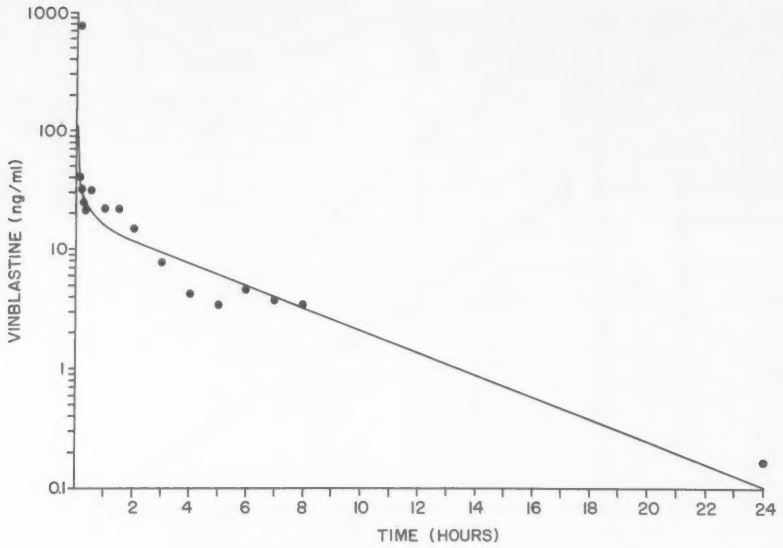

FIGURE 4.—Vinblastine levels obtained in serum after an iv bolus of 12.8 mg of vinblastine. Symbols = actual data points. Solid line = computed curve drawn by fitting the actual serum level data to an equation describing the sum of 2 exponentials.

sue found significant binding in vitro of tritium-labeled vinblastine to the microsomal-synaptosomal fraction of the tissue (21). The study suggests that, under certain circumstances, significant concentrations of vinblastine may be reached in brain tumor tissue and/or normal brain tissue. The binding of vinblastine to brain tissue might prevent the vinblastine from entering the CSF.

It is difficult to predict which chemotherapeutic agents will ultimately be found to enter the CNS in significant concentrations. The physical characteristics which are thought to permit passage of substances across the intact blood-brain barrier include low ionization at plasma pH, high lipid/water partition coefficient, and low binding to plasma proteins. On the basis of these characteristics one would have expected bleomycin to be excluded from the CSF since it is an ionized molecule with a low lipid/water partition coefficient. However, in our patient, these characteristics did not seem to limit the entrance of chemotherapeutic agents into the CNS. A recent study compared the efficacy of a water-soluble nitrosourea, ACNU, with that of two lipid-soluble nitrosoureas, CCNU and BCNU, against four animal brain tumor models (22). ACNU was as effective as CCNU and BCNU in the treatment of two of the four brain tumor models. Thus, the relative lipid solubility of the drugs was not the most significant factor in determining drug antitumor activity.

It may be possible to treat metastatic brain lesions with agents effective against the peripheral primary tumor since chemotherapeutic agents which do not ordinarily cross the intact blood-brain barrier may penetrate the CNS in the presence of tumor and/or antecedent radiotherapy. A correlation between the chemotherapy responsiveness of a metastatic brain tumor model, created by injecting Walker 256 carcinosarcoma cells into the carotid artery of Wistar rats, and the chemotherapy responsiveness of subcutaneous implants of Walker 256 carcinosarcoma cells has been demonstrated (23). In our patient the dramatic response of a primary germ cell tumor of the brain to chemotherapeutic agents which are effective against testicular germ cell tumors provides further evidence that a positive correlation exists between the responsiveness of systemic tumor and of CNS tumor of the same histologic type. Despite this positive correlation, CNS metastases can occur at a time when peripheral disease is controlled with chemotherapy. These occurrences may be due to preservation of the blood-brain barrier at the tumor margin, preventing the chemotherapeutic agents from reaching

the viable tumor cells at the edge of the metastasis. Alternatively, resistant clones of cells, selected by the chemotherapy, may be responsible for CNS metastases.

Further studies of the CNS pharmacokinetics of chemotherapeutic agents will help to improve the therapy for both primary and metastatic CNS tumors.

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Pharmacokinetics of Bleomycin After Im Administration in Man[1,2]

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SUMMARY

The pharmacokinetics of bleomycin were studied after im injection in patients with malignant lymphoma. Mean peak serum concentrations of 0.133, 0.326, and 0.587 milliunits/ml were obtained approximately 1 hour after injection of 2, 5, and 10 units/m², respectively. The mean half-life was 155 minutes, with a range of 65–235 minutes. Thirty-three percent of the administered dose was recovered in the urine after 4 hours. The urinary excretion of bleomycin was 61% during the 24 hours after injection, with some variability that was not explained by differences in dose or creatinine clearance.

[Cancer Treat Rep 65:485–489, 1981]

Study of the clinical pharmacology of the antitumor antibiotic bleomycin has been facilitated by the development of accurate, sensitive radioimmunoassays (1–3). Detailed pharmacokinetic studies following iv (4–7) and intracavitary (8) injection of bleomycin have been reported. The present report presents pharmacokinetic data following im injection of bleomycin and represents an expansion of previous preliminary data based on a smaller number of patients (7).

MATERIALS AND METHODS

The radioimmunoassay of bleomycin was performed as previously described (1). The results were confirmed in many instances by a second radioimmunoassay and in some instances by microbiologic assay as well (2,3). Except where specifically noted, the data reported in this study are those obtained with the radioimmunoassay using ^{125}I-bleomycin as the tracer (1).

Fourteen bleomycin pharmacokinetic studies were carried out in 11 patients with lymphoma after im injection of bleomycin in doses of 1–10 units/m². Only gluteal injection sites were used. Patient characteristics are listed in table 1.

Patients 4, 6, and 8 were each studied on two separate occasions. In each of these patients the first study represented the initial exposure to bleomycin, which occurred in patients 4 and 6 while they were ill with active lymphoma. The patients were re-studied 1–6 months later during clinical remission of their disease.

Serum specimens were obtained at 15, 30, 60, and 90 minutes and at 2, 3, 8, and 24 hours after bleomycin injection. Urine was collected continuously for 24 hours in fractions representing the output 0–1, 1–2, 3–4, 4–8, 8–12, and 12–24 hours after bleomycin injection. Urinary excretion of bleomycin and half-life values obtained at different dose ranges were each compared and tested for differences by a one-way analysis of variance. Area under curve (AUC) values were determined by the trapezoidal method as described by Wagner (9).

RESULTS

The pharmacokinetics following im bleomycin

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TABLE 1.—Patient characteristics

Patient No.	Study No.	Diagnosis*	Age	Sex	Creatinine clearance (ml/min)	Bleomycin dose (units/m²)
1	1	DPDL	64	Male	99	1
2	2	Richter's syndrome, chronic lymphocytic leukemia	65	Male	109	2
3	3	Hodgkin's disease	48	Male	39	2
4	4	Hodgkin's disease	32	Male	140	2
	5				132	2
5	6	Nodular histiocytic lymphoma	53	Female	89	5
6	7	Diffuse histiocytic lymphoma	62	Male	111	5
	8				—†	5
7	9	DPDL	60	Male	—‡	5
8	10	NPDL	54	Male	133	10
	11				123	10
9	12	NPDL	51	Male	57	10
10	13	NPDL	60	Male	74	10
11	14	DPDL	63	Male	100	10

*DPDL = diffuse poorly differentiated lymphocytic lymphoma; NPDL = nodular poorly differentiated lymphocytic lymphoma.
†Serum creatinine, 1.1 mg/dl.
‡Serum creatinine, 1.0 mg/dl.

administration are summarized in table 2. Figure 1 shows mean bleomycin serum concentration versus time curves for patients who received bleomycin doses of 2, 5, and 10 units/m². Peak serum concentrations were reached at a mean time of 66 ± 6 minutes after injection (range, 30–90 minutes). The maximum serum concentrations of bleomycin ranged from 0.07 to 0.70 milliunits/ml and were predictable from the dose administered. Over the dose range of 1–10 units/m², the mean peak serum concentration of bleomycin in milliunits per milliliter was 0.063 ± 0.004 times the dose in units per square meter, and it was observed approximately 60 minutes after drug administration. Drug concentrations > 0.1 milliunits/ml were maintained for 1.3, 5.5, and 9.0 hours after im bleomycin doses of 2, 5, and 10 units/m², respectively. A mean half-life of 155 ± 11 minutes was observed. An apparent tendency of the half-life to vary in proportion to the dose of bleomycin administered was not significant ($P > 0.1$). The values for AUC varied with the dose administered and ranged from 16 to 274 milliunits · min/ml.

The mean urinary excretion of bleomycin was

TABLE 2.—Pharmacokinetic parameters for bleomycin administered by im injection

Study No.	Dose (units/m²)	Serum peak (milliunits/ml)	Half-life (mins)	AUC	Urinary excretion (% of administered dose)	
					0–4 hrs	0–24 hrs
1	1	0.065	105	16	38	80
2	2	0.106	165	35	32	69
3	2	0.101	170	23	36	67
4	2	0.188	65	28	11	17
5	2	0.135	138	32	66	88
2–5*		0.133 ± 0.020	135 ± 24	30 ± 3	36 ± 11	60 ± 15
6	5	0.459	140	116	55	80
7	5	0.331	140	83	45	69
8	5	0.309	140	84	33	56
9	5	0.204	216	83	14	17
6–9*		0.326 ± 0.052	159 ± 19	92 ± 8	37 ± 9	56 ± 14
10	10	0.528	160	180	47	69
11	10	0.605	168	179	43	63
12	10	0.530	235	274	19	50
13	10	0.576	175	183	11	73
14	10	0.698	160	220	16	28
10–14*		0.587 ± 0.03	180 ± 14	207 ± 18	27 ± 7	57 ± 8

*Mean ± SE.

33.2% ± 4.8% of the administered dose during the first 4 hours and 61.1% ± 6.7% during the 24 hours following injection (fig 2). Thus, over one half of the amount of drug excreted appeared in the urine during the initial 4 hours. In 11 of the 14 studies, bleomycin was excreted most rapidly during the second hour, during which 19% of the 24-hour urinary excretion took place.

The observed negative correlation of half-life with creatinine clearance ($r = 0.593$, $P < 0.05$) results from the prolonged half-life in the two patients with the lowest creatinine clearance values. Study 3 in a patient with a creatinine clearance of 39 ml/min showed the longest half-life in the group receiving doses of 2 units/m². Similarly, study 12 in a patient with a creatinine clearance of 54 ml/min showed the longest half-life in the group receiving doses of 10 units/m². These were the only patients with abnor-

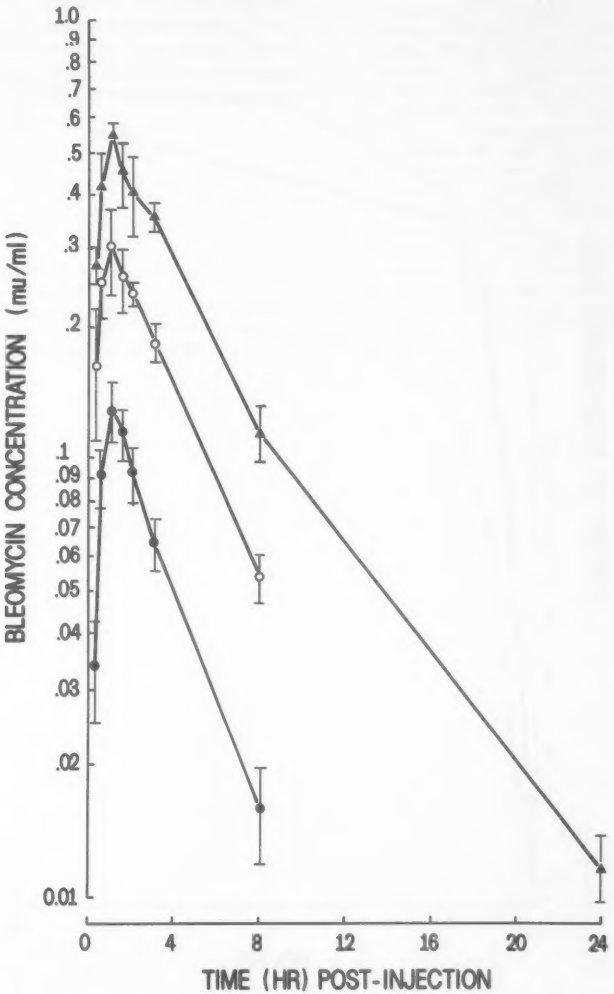

FIGURE 1.—Semilogarithmic plot of serum bleomycin concentration vs time after im administration. ▲ = 10-units/m² dose (5 studies); ○ = 5-units/m² dose (4 studies); ● = 2-units/m² dose (4 studies). Values = mean ± SEM.

mal renal function in this study. The size of the dose administered did not affect the percentage of that dose appearing in the urine ($P > 0.1$).

DISCUSSION

Bleomycin, when administered by im injection, has a half-life of 2–3 hours that is similar to the values observed after iv injection (6). The AUC and serum peak concentrations achieved varied with the dose administered. Predictable intervals were observed during which drug concentrations could be maintained above a given level; eg, serum concentrations > 0.10, 0.05, and 0.01 milliunits/ml can be maintained for 8 hours following im bleomycin doses of 10, 5, and 2 units/m², respectively (fig 1). This type of approach has recently been used to establish outpatient regimens to simulate continuous-infusion

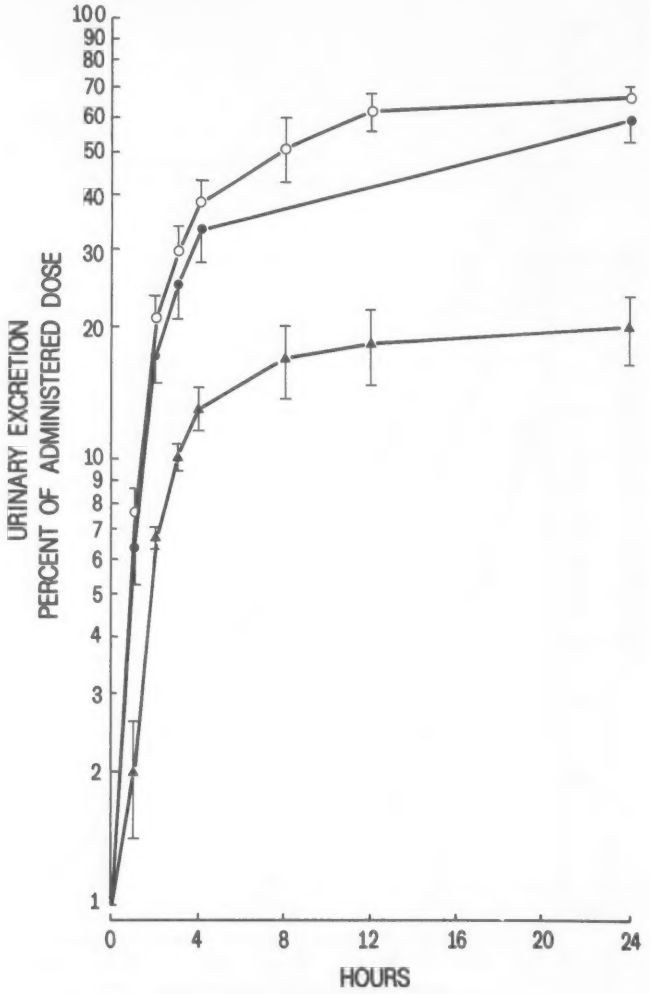

FIGURE 2.—Cumulative urinary excretion of bleomycin after im injection. ● = average of all studies (14 studies); ○ = average values for "high excretion" studies only (11 studies); ▲ = average values for "low excretion" studies only (3 studies). Values = % of total administered dose ± SEM.

regimens with intermittent im or sc administration (10,11).

Results of the urinary excretion studies fell into two groups. In 11 of the 14 studies, 69.5% ± 3.3% (range, 50%–88%) of the administered bleomycin dose appeared in the urine within 24 hours. In the three other studies, 24-hour urinary excretion of bleomycin was measured at only 20.7% ± 3.3% of the administered dose (fig 2). The reason for this variability in measured excretion of bleomycin is not readily apparent and cannot be ascribed to differences in dose, renal function, or accompanying medications. The low excretion of bleomycin noted in three patients could be due to increased tissue absorption or destruction of the drug or to excretion of a metabolite not detected with the ^{125}I-bleomycin radioimmunoassay used.

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Radiometric Enzyme-Inhibition Technique for Measuring Acivicin in Plasma[1]

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SUMMARY

A sensitive radiometric enzyme-inhibition assay is described for the determination of acivicin in plasma; it is based on the potent inhibition of carbamyl phosphate synthetase II (CPS) by the drug. Plasma is heated at 95°C for 5 minutes to quantitatively detach bound acivicin. After centrifugation, free drug is quantitated by exposing purified CPS from *Escherichia coli* to representative aliquots or subdilutions of the resultant supernatants in the presence of L-glutamine, L-aspartic acid, ATP-$MgCl_2$, $NaH[^{14}C]O_3$, and purified L-aspartate transcarbamylase (ATC) from *E. coli*. Carbamyl phosphate is first synthesized from L-glutamine, ATP-$MgCl_2$, and $NaH[^{14}C]O_3$ by the action of CPS. The unstable carbamyl phosphate thus generated is quickly and quantitatively converted to [^{14}C]carbamyl-L-aspartic acid by the action of ATC utilizing [^{14}C]carbamyl phosphate and L-aspartic acid as substrates. After a 15-minute incubation at 37°C, unreacted $NaH[^{14}C]O_3$ is dissipated at acidic pH and the newly formed [^{14}C]carbamyl-L-aspartic acid is quantitated by scintillation spectrometry. The percent inhibition of the formation of carbamyl-L-aspartic acid through the conjoint actions of CPS and ATC responds in a linear way to the logarithm of the concentration of acivicin between 20 and 200 μM. The unknown concentration of acivicin is determined indirectly by matching the percent inhibition produced by the unknown to the percent inhibition produced by a series of acivicin standards extending over the linear range. This assay is sensitive, adequately reproducible, and easy. It can be used to measure acivicin in the plasma of subjects treated with this new oncolytic agent.

[Cancer Treat Rep 65:491–494, 1981]

Acivicin is known to be a potent inhibitor of carbamyl phosphate synthetase II (CPS), the rate-limiting enzyme in the pyrimidine biosynthetic pathway (1). In addition, this antibiotic exhibits marked antitumor activity against a broad spectrum of experimental tumors (2–6). Acivicin is now undergoing phase I trials in man. In order to permit pharmacologic studies with the drug, an easy method for measuring acivicin in physiologic specimens has been developed.

MATERIALS AND METHODS

Reagents

$NaH[^{14}C]O_3$ (specific radioactivity, 59.7 millicuries/mmol) was purchased from Amersham Searle Co, Arlington Heights, IL. Aliquots of 1 millicurie/ml were dispensed into Eppendorf 1500-μl polypropylene tubes and stored at 4°C. CPS (E. C. 2.7.2.9;

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specific activity, 1.1 units/mg protein; 3.142 mg/ml of 4 M ammonium sulfate solution) purified from *Escherichia coli* by the technique of Matthews and Anderson (7) was a gift from Dr. Joseph J. Villafranca, Pennsylvania State University, University Park. Purified L-aspartate transcarbamylase (ATC) (E. C. 2.1.3.2; specific activity, 365 units/mg protein; 28.29 mg/ml of 0.04 M potassium phosphate buffer, pH 7.0) was obtained from New England Enzyme Center, Tufts University School of Medicine, Boston, MA. Acivicin was provided by the National Cancer Institute, National Institutes of Health, Bethesda, MD. All other chemicals were of the highest quality obtainable commercially.

Preparation of Plasma

Groups of four BDF_1 mice were injected ip either with acivicin (250 mg/kg) or with physiologic saline. At appropriate time intervals, mice from each group were bled from the tail vein, and the blood was centrifuged at 1000 × *g* to separate plasma. Plasma was diluted with two volumes of 0.05 M HEPES buffer (pH 7.4) and heated at 95°C for 5 minutes to quantitatively dissociate any bound acivicin. Acivicin was completely stable under these conditions. This step also inactivates any endogenous CPS and ATC which would otherwise interfere with the assay.

Preparation of ATP Reagent

The reagent consists of ATP (125 mM), $MgCl_2$ (150 mM), KCl (750 mM), L-aspartic acid (75 mM), L-glutamine (4.12 mM), and glycerol (25% vol/vol) in 0.05 M HEPES buffer, pH 7.4. After the pH was readjusted to 7.4 with 40% KOH solution, 1-ml aliquots of this mixture were dispensed into Eppendorf tubes and frozen at –87°C. Prior to the assay, 5 µl (35.6 units) of ATC from *E. coli* was added to 1 ml of the above mixture.

Measurement of CPS

For the conduct of the assay, 5-µl aliquots of ATP reagent were added to the apices of Eppendorf 1500-µl polypropylene centrifuge tubes at 4°C. Subsequently, 5 µl of appropriate dilutions of either heat-treated plasma, acivicin standards, or 0.05 M HEPES buffer (pH 7.4) was dispensed to one side of the tube, and 10 µl of CPS from *E. coli* (0.0345 unit/ml of 0.05 M HEPES buffer, pH 7.4) or 10 µl of buffer was dispensed to another side of the tube. Then, 5 µl of $NaH[^{14}C]O_3$ (5 microcuries) was dispensed on the inside of the lids and the vessels were immediately closed. The reaction was started by brief centrifugation at 12,000 × *g* and the assemblies were then incubated at 37°C for 15 minutes. The reaction was terminated by heating at 95°C for 2 minutes. After centrifugation at 12,000 × *g* for 1 minute, the lids were cut off and 50 µl of 2 N HCl was dispensed to the apices of the tubes, which were then incubated at room temperature (25°C) overnight (16 hours) in a hood to dissipate excess $NaH[^{14}C]O_3$. The tubes were immersed in scintillation vials containing 10 ml of toluene-based scintillation fluid, and the newly formed [^{14}C]carbamyl-L-aspartic acid was counted at 85% efficiency in a Beckman LS-230 scintillation spectrometer. The identification of [^{14}C]carbamyl-L-aspartic acid was carried out by loading a deproteinized reaction mixture onto a 1 × 10-cm column of Hamilton HA-X4 resin (8). The only peak unique to the supernatant prepared in the presence of CPS and ATC was carbamyl-L-aspartic acid; this peak represented about 99.5% of the radioactivity present. Under assay conditions without inhibitor, about 200,000 cpm of [^{14}C]carbamyl-L-aspartic acid are formed; blanks without CPS have about 1000 cpm.

For the computation of the CPS inhibition by acivicin, the activity of the enzyme in the presence of drug was compared to the activity in control tubes lacking it. A standard curve was established by plotting acivicin concentrations versus percent inhibition on semilog paper. Acivicin standards (1–1000 µM) were constituted in mouse plasma that was diluted and heat-denatured either prior to or immediately after addition of acivicin or in 0.05 M HEPES, pH 7.4.

Kinetic Studies

To determine the K_i of acivicin for CPS from *E. coli*, the L-glutamine concentration was varied from 0.51 to 16.5 mM. The concentrations of other substrates, cofactors, and enzymes were kept constant and were the same as previously defined. The enzyme activity was assayed as detailed earlier.

RESULTS AND DISCUSSION

Previous work in our laboratory has established that acivicin is a potent in vitro inhibitor of CPS prepared from either mammalian or bacterial sources (1). We chose to take advantage of this facet of acivicin action to devise an enzyme-inhibition assay for quantifying the antibiotic in biologic fluids. The inhibition of purified *E. coli* CPS by a series of concentrations of acivicin is illustrated in figure 1. Inhibition of enzyme activity is proportional over

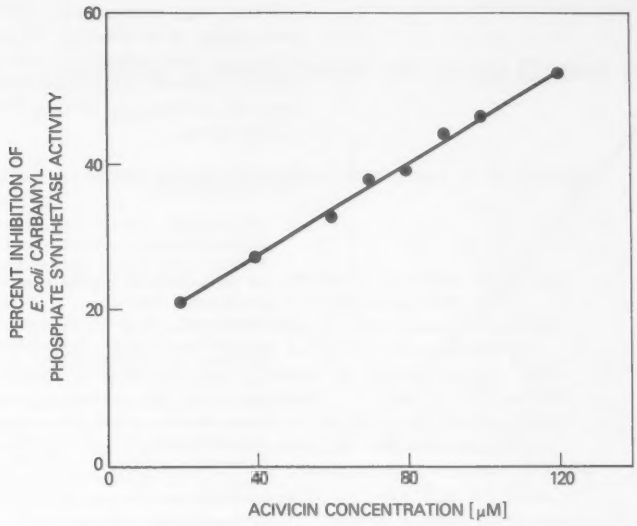

FIGURE 1.—Representative dose-response of CPS inhibition by acivicin. Various concentrations of acivicin constituted in mouse plasma were incubated with *E. coli* CPS, and the inhibition was measured as detailed in the Materials and Methods section. Values = mean of triplicate determinations; SE never exceeded 10%.

the range of 20–120 μM acivicin. The figure presented is a typical curve derived from standards prepared in diluted, heat-denatured mouse plasma. Nearly identical curves are also obtained when standards are constituted in either human plasma or 0.05 M HEPES buffer, pH 7.4. In the latter case, curves are identical whether the heating step is included or omitted, indicating that acivicin is stable to this manipulation. To explore the reproducibility of this assay, ten separate acivicin standard curves were constituted in human plasma; the mean percents of CPS inhibition (± SD) at concentrations of 50 and 100 μM acivicin were 29.0% ± 4.0% and 46.0% ± 4.0%, respectively.

To demonstrate the practical usefulness of this assay, the time course of clearance of acivicin from the plasma of mice given a therapeutic dose (250 mg/kg) of the drug was studied. It is clear from the results in figure 2 that the drug has a short plasma clearance time (half-life ~ 27 minutes) and reaches undetectable levels (< 10 μM) after 4 hours.

CPS catalyzes the synthesis of carbamyl phosphate from bicarbonate and L-glutamine, utilizing 2 mols of ATP (9). Bicarbonate binds to one subunit of the enzyme in the presence of 1 mol of ATP to form an enzyme-carboxy phosphate intermediate. Next, L-glutamine binds to the other subunit forming an enzyme-carbamyl phosphate complex. Carbamyl phosphate is released from the enzyme after binding of a second mol of ATP (9). The K_ms for ATP and L-glutamine for *E. coli* CPS are about 25 and 1 mM, respectively (9). Comparable substrate concentrations are used in the present assay. Acivicin competitively inhibits the utilization of L-glutamine by this enzyme with a K_i of 2.0 μM (data not shown). Since acivicin is an antagonist of L-glutamine, the concentration of L-glutamine can influence the inhibitory potency of the drug. This point assumes practical relevance when it is recalled that parenteral administration of acivicin to mice provokes an expansion of the L-glutamine pools (1). Additionally, circulating levels of L-glutamine are quite high (~ 500 μM in most mammalian species [D. A. Cooney and H. N. Jayaram, unpublished observations]). Under the conditions described, however, this assay is not compromised by plasma L-glutamine content. The initial threefold dilution of plasma samples during preparation as well as the fivefold dilution in the assay level serves to minimize this potential source of interference.

Although the theoretic sensitivity of this assay should approximate the K_i of acivicin for the enzyme, the sensitivity under the conditions described is about tenfold less. Sensitivity approaching this

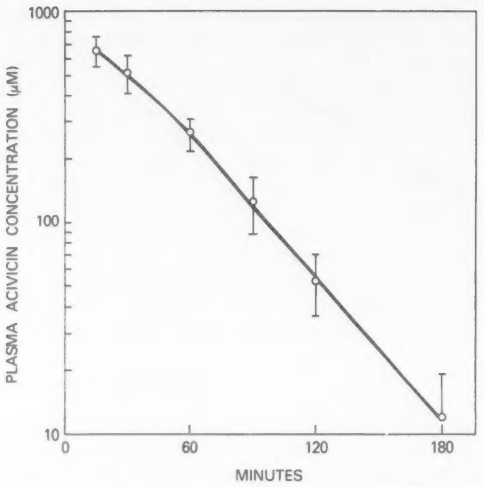

FIGURE 2.—Time course of clearance of acivicin from mouse plasma. Male BDF_1 mice (25 g) were injected ip with 250 mg/kg of acivicin; 25 μl of heparinized blood was drawn from the tail vein at various time intervals and was processed as detailed in the Materials and Methods section. Values = mean ± SE of triplicate determinations done on individual samples obtained from 4 mice. Assay variability (SE) for the determination of acivicin concentrations in plasma from individual mice never exceeded 10%.

limit can be obtained by reducing assay L-glutamine concentrations about fourfold to fivefold; however, precision is sacrificed. A final concentration of < 1 mM L-glutamine progressively reduces the number of counts generated. Attempts to improve sensitivity were made by regulating the order of addition. 6-Diazo-5-oxo-L-norleucine, another L-glutamine antagonist, binds irreversibly to its target enzymes (10). If this were the case with acivicin, a 30-minute preincubation of acivicin samples or standards with CPS prior to the additions of L-glutamine might enhance the percent inhibition; in fact, this approach was without effect.

Using this quick, easy, and reproducible assay procedure, it is possible to quantitate acivicin in plasma at concentrations > 10–20 μM. We are now ready to assess the pharmacokinetics of acivicin in patients undergoing clinical trials with this new oncolytic drug.

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Disposition of Thymidine Administered as Large Doses to Rats and Mice[1,2]

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SUMMARY

Blood and urine levels of thymidine and its catabolic product, thymine, have been determined for mice and rats given a single large dose of thymidine and for rats during and after infusion of large amounts of this drug. For mice given a bolus dose (400 mg/kg, 1.2 g/m^2), two phases of elimination of thymidine from blood were evident, an initial phase (half-life = 4 mins) and a longer second phase (half-life = 17 mins). The initial (2-min) level in blood was 2.8 mM. For rats given an equivalent dose (150 mg/kg, 1.2 g/m^2), three phases with half-lives of 2, 29, and 365 mins were observed. The initial (5-min) concentration in the blood was 0.6 mM. More of a dose of [2-^{14}C]thymidine was converted to CO_2 by rats than by mice. For both species, small amounts of radioactivity from this labeled compound became associated with macromolecules of the small intestine. Following infusion of rats with thymidine at rates of 300 and 600 mg/kg/hr for 24 hrs (60 and 120 g/m^2, respectively), steady-state blood levels were approximately 0.7 and 1.5 mM, respectively. When the infusions were stopped, a phase with a half-life of 35 mins and a longer phase of indeterminate length were noted for each dose. Elimination of metabolically formed thymine from the blood of rats and excretion into the urine was mediated by a process that was apparently saturated.

[Cancer Treat Rep 65:495–499, 1981]

Based on experiments in which continuous infusion of large amounts of thymidine resulted in inhibition of growth of xenografts of a variety of human tumors implanted in nude mice (1–4), thymidine is presently undergoing clinical trials as an anticancer agent (5,6). The biochemical mechanism of action for thymidine is that a metabolite, thymidine triphosphate, inhibits ribonucleotide reductase, which causes a depletion of the pool of deoxycytidine triphosphate and subsequent disruption of DNA synthesis (7–9).

The objective of this study was to determine the disposition of thymidine given as large doses to mice and rats. Specifically, we sought to determine the disposition of this agent and of thymine, a catabolic metabolite, after large bolus doses and during and after infusion.

MATERIALS AND METHODS

In the administration of bolus doses, thymidine (Sigma Chemical Co, St. Louis, MO) was dissolved in distilled water at a concentration of 40 mg/ml and was injected into the tail veins of male BDF$_1$ mice at a dose of 400 mg/kg and into the tail or femoral veins of male Sprague-Dawley rats at a dose of 150 mg/kg. Both doses are equivalent to 1.2 g/m^2. The mice weighed 20–25 g and the rats weighed 250–500 g. At various time intervals, blood was obtained from the unused femoral vein of the rats. Mice were

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killed by decapitation and blood was collected in heparinized beakers.

To determine the metabolism and excretion of [2-^{14}C]thymidine, the drug, having a specific activity of 30 microcuries/mmol was injected similarly, and the mice and rats were placed immediately in metabolism chambers connected to scrubber flasks containing 2 N NaOH. The amount of $^{14}CO_2$ produced was determined by analysis of a 1-ml portion of the NaOH solution at 24 hrs after injection. To the sample, 4 ml of water and 10 ml of Scintiverse (Fisher Scientific Co, Fairlawn, NJ) were added, and the preparation was counted in a liquid scintillation spectrometer equipped with an external standard. Urine and feces were collected, and portions were solubilized in Soluene 350 (Packard Instrument Co, Downers Grove, IL) and counted for radioactivity after addition of 15 ml of Liquiflor (New England Nuclear Corp, Boston, MA). After 24 hrs, the animals were removed from the chambers and killed by exsanguination. The lungs, kidneys, liver, and either 30 cm (for rats) or 15 cm (for mice) of proximal ileum were removed from each animal, blotted on filter paper, and placed on dry ice. Prior to freezing, the intestines were scraped and rinsed in 0.9% NaCl. To determine incorporation into macromolecules, the tissues were thawed, weighed, and homogenized in four volumes of water with a Brinkmann Polytron equipped with a P-10 generating head (Brinkmann Instruments, Westbury, NY). Portions (0.5 ml) of each preparation were added to 5 ml of 10% trichloroacetic acid, and the suspension was homogenized as described above. After centrifugation at 2000 × *g* for 10 mins, the insoluble material was collected and washed three times with 10% trichloroacetic acid and once with water. For each wash, the Polytron was used to resuspend the precipitate. The washed precipitate was dissolved in 1 ml of Soluene 350 and counted for radioactivity after addition of 15 ml of Liquifluor. In studies with [2-^{14}C]thymidine, data were obtained from four individual rats and four groups of two mice.

For infusion, rats were anesthetized, restrained on a board, and fitted with jugular catheters (10). A 22-gauge Teflon catheter was inserted into a lateral tail vein and connected to an infusion syringe by means of a polyethylene tube. An external urinary catheter was inserted. After the rats had recovered from the anesthesia, the infusion pump was adjusted to deliver 10 mg/ml of thymidine, dissolved in 0.9% NaCl, at a rate of 300 or 600 mg/kg/hr. The infusion lasted for 24 hrs, giving total doses of 60 or 120 g/m^2, respectively. Blood (0.2 ml) and urine samples were collected at various times during and after infusion. The jugular catheter was kept open by rinsing with heparinized saline after collection of each blood sample.

Analysis of thymidine and thymine was accomplished in a manner similar to that reported by Zaharko et al (11). Blood was homogenized in five volumes of 0.5 M perchloric acid with a Polytron. After centrifugation, the supernatant was mixed with one-half volume of 1 M KOH. The neutral supernatant, obtained after further centrifugation, was injected into a high-pressure liquid chromatograph (Waters Associates, Milford, MA) equipped with a Hewlett-Packard 3380 integrator. Urine samples, without treatment, were injected directly into the instrument. The column was an ODS-II (Whatman Inc, Clifton, NJ), and the eluting solvent was water:acetonitrile (95:5, vol/vol). Eluting materials were detected by UV adsorption at 254 nm. Retention times were 8.5 mins for thymine and 12.5 mins for thymidine.

Binding of thymidine to plasma proteins was determined by filtration of plasma containing various concentrations of [^{3}H]thymidine through Pellicon membranes fitted into 13-mm ultrafiltration cells (Millipore Corp, Bedford, MA).

Data regarding the blood concentrations of thymidine were processed using a modified form of NONLIN (12) and CSTRIP (13). For postinfusion experiments, the blood concentration at zero time was set at 0.00001 hr since the program did not accept zero for the time. The data were fitted to one-, two-, and three-compartment open models. A model was accepted as the best fit if an additional term, or compartment, failed to reduce significantly the weighted sums of squares.

RESULTS AND DISCUSSION

Prior to initiation of infusion experiments, the disposition of thymidine was determined following bolus iv injections into mice (400 mg/kg) and rats (150 mg/kg). In mice, thymidine was eliminated from the blood in two phases with half-lives of 4 and 17 mins (fig 1A). The concentration of the metabolite, thymine, increased for about 20 mins and then decreased, apparently in a linear (nonlogarithmic) fashion for the following 100 mins.

In another study involving mice injected iv with approximately 25 mg/kg (14), an initial phase of distribution with a half-life of about 1 min was evident for 5 mins after dosing. This was followed by a second phase, lasting about 30 mins, with a half-life of 8 mins. The initial phase that we observed following a dose of 400 mg/kg had a half-life of 4 mins, which is intermediate to those for the smaller dose (14). Another half-life value reported for mice injected

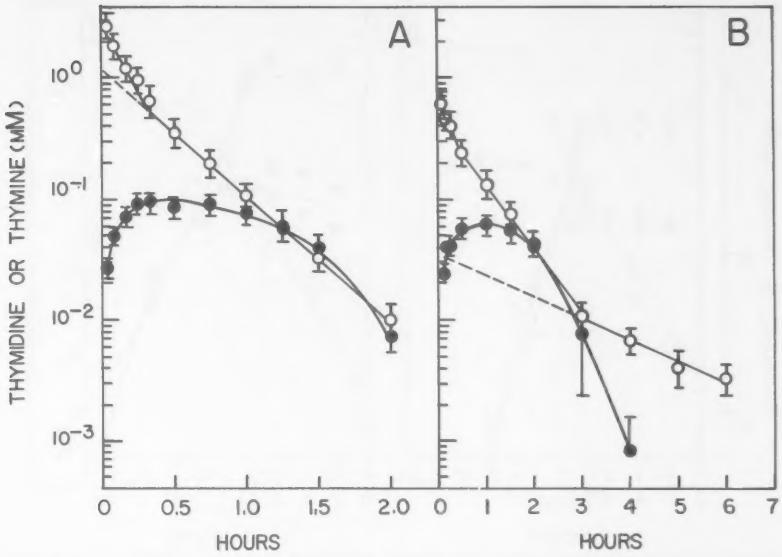

FIGURE 1.—Elimination of thymidine (○) and its metabolite, thymine (●), from the blood of mice (A) and rats (B) following a single iv dose of 1.2 g/m². Blood from 3 groups of 3 mice each and from 4–7 rats was assayed for each point. Values are shown with SE.

iv with thymidine is 9.4 mins (15). In this case, the dose was about 5 mg/kg. In mice, thymidine at low doses apparently has a shorter half-life. We have not studied the kinetics of such low, nontherapeutic doses.

For mice injected with [2-^{14}C]thymidine given as a bolus dose, 16% ± 2% (mean ± SD) of the dose was recovered in 24 hrs as $^{14}CO_2$. In the urine, 43% of the dose was unchanged drug, 12% was thymine, and 14% was an unidentified metabolite. Less than 0.5% was recovered in the feces. The macromolecules of the small intestine contained 28 ± 2 μg-equivalents of radioactivity/g of tissue, and liver contained 7 ± 1 μg-equivalents/g. Less than 1.0 μg-equivalent/g was found in lung and kidney.

In rats, phases with half-lives of 2 (?), 29, and 365 mins were observed following the bolus dose (fig 1B). With the determined values for blood levels of thymidine in these rats, the existence of the first phase could not be established unequivocally by statistical means. From data derived with rats injected intra-arterially with about 20 mg/kg of thymidine (16), we have calculated a half-life value of 25 mins, which compares favorably with our value of 29 mins for the second phase. In rat blood, the level of thymine increased for 1 hr and then dropped rapidly in a manner similar to that for the mouse (fig 1B).

For rats injected with a bolus dose of [2-^{14}C]thymidine, 49% ± 2% of the dose was recovered as $^{14}CO_2$ in 24 hrs. In the urine, 30% of the dose was present as thymidine, 13% as thymine, and 2% as an unidentified metabolite. Less than 0.5% of the dose was recovered in the feces. Macromolecules of the small intestine of rats contained 34 ± 2 μg-equivalents of radioactivity/g of tissue. The amounts in liver, lung, and kidney were 7 ± 1, 5 ± 2, and 3 ± 0 μg-equivalents/g, respectively. As compared to mice, rats converted more of the dose to CO_2 and less to the unidentified metabolite. For both species, the small intestine, which contains numerous proliferating cells, incorporated more of the radioactivity into macromolecules.

The profile of thymidine in the blood of rats during and after infusion of 300 mg/kg/hr is shown in figure 2A. Between 1 hr after the infusion started and 24 hrs, when it was stopped, the blood level of thymidine remained practically constant at 0.7 mM. Following the infusion, thymidine disappeared from the blood, with a half-life of 36 mins, a value similar to that for the second phase following a bolus dose (fig 1B). Figure 2B shows the content of thymidine in the blood during and following infusion of 600 mg/kg/hr. For this higher dose, the concentration of thymidine in the blood during infusion was approximately double (1.5 mM) that for the lower dose. Following the infusion, loss of thymidine from the

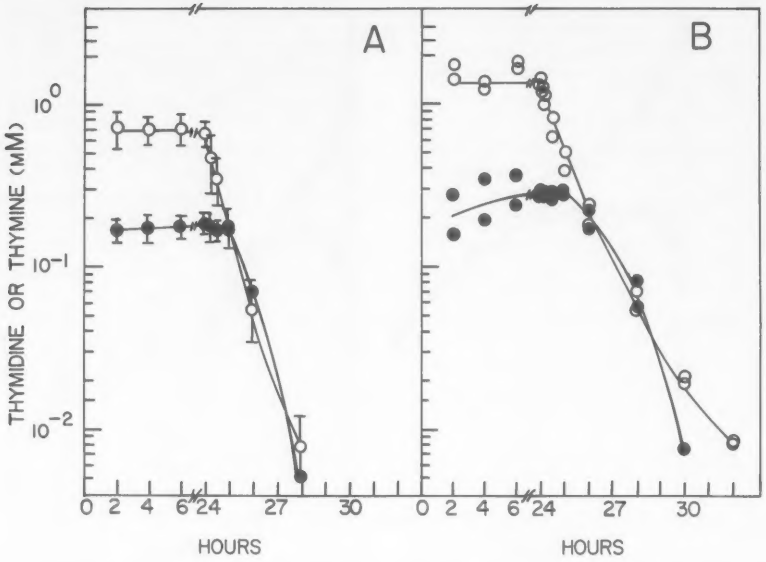

FIGURE 2.—Blood levels of thymidine (○) and thymine (●) in rats infused for 24 hrs with 300 mg/kg/hr (A) or 600 mg/kg/hr (B) of thymidine. For each point in (A), blood from each of 3 rats was assayed, and values are shown with SD. In (B), values are shown for 2 rats.

blood occurred, with a first phase having a half-life of 34 mins and a second phase, the length of which could not be adequately determined.

The fact that the elimination half-life was the same after infusion as after a bolus dose indicates that no process affecting these values was saturated by the infusion. Further, the rapid elimination, following infusion, of most of the thymidine from the body of the rat indicates that no substantial "third compartment" exists. Had such a large compartment existed, excretion would have been slower after the infusion. The minor third phase observed in some of the studies reported here apparently is not due to serum protein binding, for at concentrations between 0.5 and 100 μg/ml, < 5% of the thymidine present was bound to blood proteins (not shown).

The half-life for elimination of thymidine from the blood of man ranges from 7.5 to 106 mins (5,6,11,17), increasing with the size of the dose administered (6,11). Our experiments with rats, however, did not demonstrate a longer half-life for thymidine with the higher dose of the two given. It is possible that the half-life in rats would change if substantially larger (or smaller) doses were administered.

Concentrations of the metabolite, thymine, in the blood of the infused rats are also shown in figures 2A and 2B. For both doses, the levels of this compound remained essentially constant during the infusion. Afterward, the levels decreased, but only after 1 hr. A delayed decrease in the blood concentration of thymine following cessation of infusion, as noted for rats (fig 2), has also been observed for man (5,11). When the points obtained after 1 hr (fig 1) and after stopping the infusion (fig 2) were plotted on rectilinear paper, straight lines resulted, indicating the presence of a saturated elimination process.

After the infusion of thymidine into rats was stopped, its elimination in the urine of those given 300 mg/kg/hr declined exponentially with a half-life of 40 mins (fig 3A). Elimination of thymine in the urine followed the same pattern as noted for elimination from the blood, again suggesting the involvement of a saturated process. However, there appeared to be more thymine in the urine following the high dose. Apparently, the metabolism of thymidine was not saturated by these doses. Thymidine excretion in the urine of a single rat infused with 600 mg of thymidine/kg/hr (fig 3B) was similar, although in greater amounts, to that for the lower dose. The half-life for excretion of the large dose of thymidine was 36 mins.

Over the entire 32-hr period, 80% ± 12% and 16% ± 1% (mean ± SD) of the low dose were recovered in the urine as thymidine and thymine, respectively.

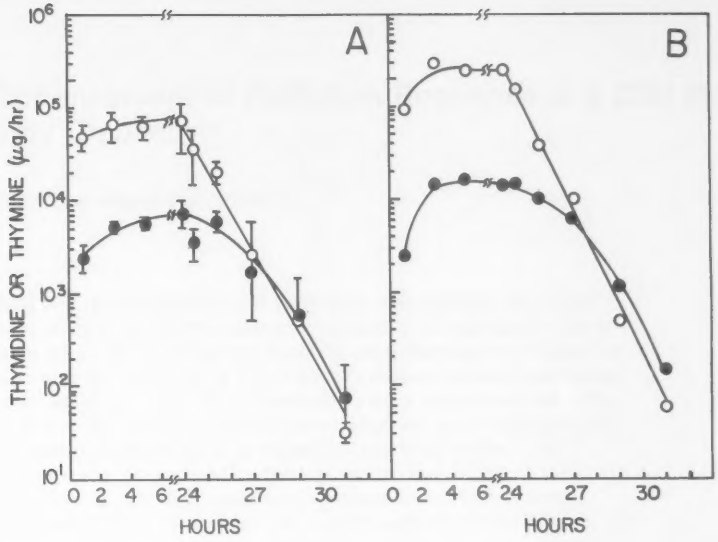

FIGURE 3.—Elimination of thymidine (○) and its metabolite, thymine (●), in the urine of rats infused for 24 hrs with 300 mg/kg/hr (A) or 600 mg/kg/hr (B) of thymidine. For each point in (A), urine from each of 3 rats was assayed, and values are shown with SD. In (B), values are shown for 1 rat.

For the high dose, the corresponding values were 93% and 15%.

These studies demonstrate that following bolus doses of thymidine to mice and rats and following infusion of this drug to rats, both thymidine and its metabolite thymine are rapidly eliminated from the blood. At least in rats, the compounds are excreted rapidly in the urine. Continuous administration appears to be necessary to achieve sustained, high concentrations of thymidine in the blood of experimental animals.

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Selective Enhancement of Radiation Response in a C3H Mammary Carcinoma by Cisplatin[1,2]

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SUMMARY

The interaction between cisplatin and radiation was studied in a C3H mammary carcinoma and its surrounding normal skin. Treatment with 6 mg/kg of cisplatin given 30 mins before irradiation enhanced the radiation response in the tumor by a factor of 1.7, whereas the same drug treatment given immediately after or 4 hrs after irradiation only enhanced the effect by a factor of 1.2–1.3. No interaction between radiation and cisplatin was observed in the surrounding skin. It is hypothesized that in the C3H mammary carcinoma the prominent radiation-modifying effect of cisplatin given before radiation is due to a radiosensitization involving hypoxic tumor cells combined with a tumor cell-destructive effect of the drug itself.

[Cancer Treat Rep 65:501–503, 1981]

In the search for a better cancer treatment, the use of a multimodal therapeutic strategy has become more and more apparent. Thus, many cancer chemotherapeutic agents have been investigated with regard to their ability to enhance the effect of ionizing radiation in solid tumors in order to improve local and regional cancer treatment.

Although such an effect can be demonstrated in cell cultures, it must be realized that when applied to experimental tumor and normal tissue systems or in clinical treatment, the therapeutic gains obtained are doubtful. This is primarily because the drugs that have an enhancing effect on the radiation response in tumors also frequently sensitize the radiation reaction in normal tissues to a similar or even greater degree. However, the present data on the interaction between cisplatin and radiation indicate that this drug has an ability to selectively increase the radiation response in a C3H mammary carcinoma without enhancing the radiation damage to the surrounding normal tissue.

MATERIALS AND METHODS

The tumor system and treatment technique have recently been described in detail (1). In short, a C3H mammary carcinoma transplanted into the feet of C3D2F$_1$/Bom mice was treated with single doses of radiation using a conventional 250-kv orthovoltage unit with a dose rate of 1.9 Gy/min. Cisplatin was given as a single ip injection at a dose of 6 mg/kg before or after irradiation. Treatment was given to tumors with a volume of 200 mm^3 calculated by the formula $D_1 \times D_2 \times D_3 \times \frac{\pi}{6}$, where D_1, D_2, and D_3 are three orthogonal diameters.

The effect of cisplatin alone was evaluated as the mean time needed for tumors to increase in volume from 200 to 1000 mm^3 and was compared to untreated controls.

The interaction between cisplatin and radiation treatments was studied in both the tumor and the surrounding skin on the tumor-bearing foot.

The tumor response was calculated in terms of the radiation dose that induces local control at 120 days after treatment in 50% of the animals (TCD_{50}).

The normal tissue end point was the dose of radiation needed to achieve full moist desquamation

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on the irradiated foot within 30 days in 50% of the animals (DD_{50}). The TCD_{50} and DD_{50} values were calculated by the logit method from assays with animals allocated into six to ten dose groups (1).

RESULTS

The effect of cisplatin on tumor growth is shown in table 1. A single ip dose of 6 mg/kg caused a 75% increase in mean growth time in this C3H mammary carcinoma.

The response to the combined radiation and drug treatments is shown in table 2. Application of the drug 30 mins before irradiation resulted in a strong enhancement of the radiation effect in the tumor as evidenced by a decrease in TCD_{50} from 56.3 to 32.8 Gy, resulting in an enhancement ratio of 1.7. Administration of the drug 30 mins after irradiation also resulted in an enhancement of the tumor response, but with a lesser enhancement ratio of about 1.3. A similar effect was observed when cisplatin was given 4 hrs after irradiation. It was, however, remarkable that none of these combined treatment schedules caused any enhancement of the radiation response in the surrounding skin. Thus, all treatment regimens resulted in a therapeutic gain which was by far most pronounced if cisplatin was applied immediately before irradiation.

DISCUSSION

Such marked and selective enhancement of the radiation effect in a solid tumor has not previously been described among cancer chemotherapeutic drugs that are known to interact with radiation therapy. The mechanism of this interaction is not fully known, but it is likely that the enhancement observed with preradiation drug therapy is, at least in part, due to a radiosensitization of hypoxic tumor cells with cisplatin. An enhancement ratio of 1.7 in the present tumor, which contains approximately 10% hypoxic cells, can only occur if a significant proportion of the hypoxic cells are either destroyed by the drug or sensitized for the effect of radiation. The latter effect has been shown in bacterial and cellular systems in vitro (2–5) and may be an explanation of the observed findings. However, the fact that cisplatin given after irradiation also caused an increased tumor response, although to a smaller degree, may be due either to a direct tumor effect of the drug itself or to a reduced repair of sublethal radiation damage. Although the latter effect has

TABLE 1.—Effect of single ip dose of cisplatin on growth of a C3H mammary carcinoma

Treatment	No. of animals	Tumor growth time (days)*	Increase in growth time
None	10	6.5 ± 1.2	—
Cisplatin (6 mg/kg)	7	11.4 ± 1.5	75%

*Values = days needed for tumor to grow from 200 to 1000 mm^3 (mean ± SD).

TABLE 2.—Effect of treatment sequence on the interaction between cisplatin and radiation therapy in a C3H mammary carcinoma and its surrounding skin

Treatment	No. of animals	Tumor response		Skin response		Therapeutic gain factor†
		TCD_{50} (Gy)	Enhancement* ratio	DD_{50} (Gy)	Enhancement ratio	
Radiation alone (control)	91	56.3 (55.0–57.7)‡	—	31.8 (28.9–34.8)	—	—
Cisplatin 30 mins before irradiation	72	32.8 (28.9–37.3)	1.72 (1.54–1.91)	32.9 (30.7–35.3)	0.96 (0.88–1.04)	1.79
Cisplatin 30 mins after irradiation	78	44.1 (39.5–49.3)	1.28 (1.16–1.40)	32.8 (28.9–37.1)	0.97 (0.88–1.07)	1.32
Cisplatin 4 hrs after irradiation	54	45.9 (41.6–50.8)	1.23 (1.14–1.32)	32.9 (30.8–35.1)	0.97 (0.89–1.05)	1.27

*Enhancement ratio: $\frac{\text{dose of radiation alone needed to achieve end point}}{\text{dose of radiation combined with cisplatin needed to achieve end point}}$.

†Therapeutic gain factor: $\frac{\text{enhancement ratio for tumors}}{\text{enhancement ratio for normal tissue}}$.

‡Values in parentheses = 95% confidence limits.

been described in cells exposed to different platinum complexes (6–8), it is probably of minor importance in the present system, since application of cisplatin 30 mins or 4 hrs after radiation did not result in any significant difference in the TCD_{50} or DD_{50} values. Furthermore, administration of 6 mg/kg of cisplatin without irradiation resulted in a 5-day growth delay in the C3H mammary carcinoma, which indicates some degree of drug-induced damage to the tumor. Thus, the effect of cisplatin given after irradiation is more likely to be a tumor-destructive effect of the drug itself independent of the radiation response (ie, an additive effect).

The significantly larger enhancement observed when cisplatin was given prior to irradiation was more than an additive effect. Therefore, this interaction is likely to be explained by a cisplatin-induced radiosensitization.

In the present tumor, the TCD_{50} value is almost solely dependent on the radiosensitivity of the hypoxic fraction. Such a radiosensitizing effect must therefore include a sensitization of hypoxic cells similar to that observed in vitro (2,5).

A similar interaction between cisplatin and radiation therapy has recently been described in other experimental tumors, using growth delay as the end point (2,9–11). Apparently, the degree of this enhancement is widely varying, although the difference in experimental design excludes a more detailed quantitative comparison. The lack of any radiosensitization in the surrounding skin may not be a general phenomenon in all normal tissues, since some radiosensitizing effect of cisplatin is described in especially rapidly proliferating tissues such as those of the intestine (7,12). However, the magnitude of this normal tissue radiosensitization is considerably smaller than the enhancement ratio observed with preirradiation drug treatment in the present mammary carcinoma.

Much emphasis is currently placed on the development and use of hypoxic cell radiosensitizers in an attempt to improve the effect of radiotherapy. This is because radioresistant hypoxic cells are considered to be one of the major hurdles to achieving a satisfactory local response in many cancers (11,13). The apparent hypoxic cell radiosensitizing ability of cisplatin together with its well-known activity against several solid tumors (14) gives it a potentially important role in multimodality therapy regimens. Thus, the use of cisplatin in conjunction with radiotherapy deserves further experimental investigations in more clinically related fractionated treatment schedules.

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Re-evaluation of the Combination of CCNU, Vincristine, and Bleomycin in the Treatment of Malignant Disseminated Melanoma[1,2]

Brief Reports and Preliminary Communications

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In 1976 we reported the results of the combination of CCNU, vincristine, and bleomycin in the treatment of disseminated malignant melanoma (1). An unusually high response rate of 40% was detected in a series of 42 patients. Similar results were published later for 13 patients treated at the Royal Marsden Hospital, London, England (2). After publication, the trial continued and patients with disseminated melanoma were routinely treated with the same regimen by several different physicians at our Institute. We report the final results on all the patients treated with this combination.

Since 1976 the treatment schedule has not changed. CCNU (130 mg/m^2) was given orally on a 6-week basis and vincristine (maximum unit dose, 2 mg) and bleomycin (maximum unit dose, 15 mg) were given iv every week for 6 weeks. In case of an objective response, this treatment was given until evidence of disease progression.

Subsequent to 1976, 25 patients were entered in the trial, for a total of 67 patients. Data of the entire series were independently reviewed and the same standard criteria for responses were used (1). We excluded 18 patients from the study, mainly because of major protocol violations that were made when this combination was given by several staff members at the Institute. In the 49 fully evaluable patients, survival and overall response duration were calculated from the day of entry in the trial.

The previous responses could be confirmed, but this three-drug combination failed to achieve additional responses since 1976. Summarizing, the response rate to our combination, when taking into account the totality of the trial, decreases from 48.5% in fully evaluable patients to 35%, or from 40% to 25% for the total number of patients entered.

The 95% confidence intervals of the early and final results overlap, both for the total number of patients and for the fully evaluable patients only. The difference between the 40% response rate noted in the first series and the 0 response rate noted in the subsequently treated patients is highly significant ($P < 0.001$).

The mean survival time of all patients was 42 weeks (SE, 6); one patient with hepatic metastases is still in remission at 209+ weeks after treatment initiation. The mean duration of the overall response (17 patients) was 36 weeks (SE, 13). The complete response duration (five patients) was calculated from the date the complete remission was recorded to the date of disease progression, with a particularly long mean of 61 weeks (SE, 28).

To explain the difference in treatment results before and after 1976, a multivariate statistical analysis was conducted for the 13 patients, following variables of potential prognostic influence (table 1). The most significant imbalances before and after 1976 did not appear in the variables that were found to be of prognostic influence on response; these imbalances do not seem sufficient to fully explain the drastic difference in treatment results. Furthermore, it should also be noted that the total amount of drug given to patients did not differ before and after 1976.

DISCUSSION

Positive results may be misleading because they are often reported early and cannot always be confirmed in larger studies. The rate of false-positive errors (delta errors) (3) is important in small clinical series. This type of error depends, to a large extent, on the sample size. For example, the high activity of the BACON (bleomycin, doxorubicin, CCNU,

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TABLE 1.—Prognostic factors of the 2 series

Patient characteristics	Treatment given— Before 1976 (30 patients)	After 1976 (19 patients)
Mean age at entry in the study	50.4 yrs	47.8 yrs
Mean disease duration before entry in the study	102 wks	103 wks
Sex (M/F)	10/20*	10/9
Main tumor location (limbs/head/trunk)	16/7/7	7/6/6
Prior chemotherapy (yes/no)	11/19	2/17
Prior immunotherapy (yes/no)	14/16	6/13
Transit lesions (yes/no)	4/26	7/12
Skin lesions (yes/no)	25/5	7/12
Node lesions (yes/no)	11/19	10/9
Hepatic lesions (yes/no)	5/25	4/15
Pulmonary lesions (yes/no)	10/20	10/9
CNS lesions (yes/no)	3/27	2/17
Bone lesions (yes/no)	3/27	1/18

*Values = No. of patients.

Oncovin, and mechlorethamine) regimen for squamous cell lung cancer, developed at the M. D. Anderson Hospital and Tumor Institute, Houston, TX, by Livingston et al (4) (45% response rate among 38 patients with extensive disease), could not be confirmed by the Southwest Oncology Group (25% response rate among 98 fully evaluable patients) (5). Since our combination was routinely administered after 1976 by several different physicians, a situation similar to that of a cooperative group may have existed. We have shown that there was no significant difference in patient selection or in treatment administration. We therefore think that the delta error and the variation of the sample size are probably sufficient to explain the decrease in the response rate that we observed in our series.

The advantages of this combination of CCNU, vincristine, and bleomycin in the treatment of disseminated malignant melanoma seem to be only moderate in terms of survival, with the possible exception of a few patients achieving a complete response. A high risk:benefit ratio and a severe cost in toxicity were already shown (1). Because of the absence of further responses, medical staff discouragement became evident when treating patients with melanoma and prompted the present global reevaluation.

This combination is no longer used at the Jules Bordet Institute. Patients with metastatic malignant melanoma are now treated with investigational agents, as first-line chemotherapy, according to phase II cooperative trials of the European Organization for Research on Treatment of Cancer.

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Misuse of Scalp Hypothermia[1]

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Brief Reports and Preliminary Communications

SUMMARY

Scalp hypothermia has been introduced to reduce the temporary epilation associated with certain cytotoxic drugs. This has improved compliance with drug delivery for some patients. It is currently not recommended for use in those tumors with a high prevalence of scalp metastasis, ie, leukemia and lymphoma. We have treated a patient for mycosis fungoides who demanded use of a "cooling cap" while undergoing consolidation chemotherapy. Cutaneous disease recurred on the scalp, with no other evidence of mycosis fungoides. Caution should be used in reducing drug delivery to the scalp while treating tumors manifesting cutaneous stem cell tumor nests.

[Cancer Treat Rep 65:507–508, 1981]

Recently, a hypothermic scalp device ("cooling cap") to reduce the incidence and severity of alopecia in patients receiving chemotherapy has been available (1,2). Such a device is generally used for patients with solid tumors treated with alkylating agents well known to cause significant alopecia (4–7). Although this side effect of chemotherapy may appear inconsequential compared to other significant side effects such as pancytopenias, muscle weakness, and heart damage, we have found that providing such a service has improved the sense of well-being in some patients.

We report here a case of a tumor recurrence on the scalp in a man who decided to use such a hypothermic scalp device while receiving systemic chemotherapy for mycosis fungoides (MF). This was against the advice of all physicians involved with his case.

The patient was a 52-year-old white male who presented in 1978 with a 4-year history of erythematous and itchy skin on exposure to sun or physical stress. Biopsy of a macular scaly, plaque chest lesion confirmed MF. Since the disease was limited to the skin he was given 125-kvp X-ray treatment to affected lesions on the chest, scalp, and nape of the neck. A dose of 3600 rads to a depth of 5 mm was delivered over 50 days. At the completion of X-ray therapy, 10 mg of mechlorethamine dissolved in 60 ml of water was applied topically to the entire skin surface every other day. A 2-cm firm supraclavicular node developed, the biopsy of which was dermatopathic lymphadenopathy, with no evidence of MF. Extension of cutaneous disease to the chest and neck necessitated a further 2850 rads of 125-kvp orthovoltage to a depth of 5 mm. In January 1979, biopsy-proven MF arose between the toe webs of the right foot, presenting as a violaceous plaque with central ulceration. Plaque disease progressed in the feet, chest, and inguinal fossa despite the use of topical mechlorethamine in a 1:4 dilution. In September 1979, electron-beam therapy was begun. A total of 3500 rads was delivered over 60 days by whole-skin 6-mev electron beam. A boost of 1500 rads by 125-kvp orthovoltage was delivered over 60 days to the perineum and 1000 rads of 125-kvp orthovoltage was delivered over 16 days to the soles of the feet. Complete clinical resolution resulted.

After a 1-month rest, systemic chemotherapy was begun. The following treatment program was delivered monthly on an outpatient basis: doxorubicin at a dose of 20 mg/m² iv; semustine at a dose of 50 mg/m² orally; and cyclophosphamide at a dose of 200 mg orally daily × 5 days.

Due to fear of permanent alopecia, the patient was adamant that a hypothermic scalp device be used. After 3 monthly cycles using the cooling cap, a scaly erythematous occipital scalp plaque developed which was proven by biopsy to be MF. There was no evidence of other visceral or cutaneous disease. He has since stopped using a hypothermic cooling device with gradual resolution of the scalp plaque without a change in chemotherapy. There have been no other complications of drug therapy.

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DISCUSSION

MF is a cutaneous T-cell lymphoma with the neoplastic T cell demonstrated to have helper-cell activity (8,9). Long-term remissions are generally limited to patients with early cutaneous (stage I and II) disease (10). Current standards of treatment for advanced cutaneous T-cell lymphoma at this center make use of 3000–3600 rads of total-body electron-beam therapy followed by 6 months of systemic cytotoxic chemotherapy in an attempt to eliminate all tumor burden. This has led to a therapeutic response ranging from stabilization of disease, to complete disappearance of all disease.

The cytotoxic agents used to treat MF have been associated with reversible, but severe, alopecia (6,11,12). At the time of referral to our department all patients have undergone whole-skin electron-beam therapy (13) and have complete alopecia. Therapy with cytotoxic drugs is begun 1 month after the completion of irradiation at a time when hair growth has begun again. This patient was acutely aware of change in body image, and with the improvement in skin disease was adamant in his demand to lose no more hair.

Dean et al (2) have shown the reduction of doxorubicin-induced alopecia by scalp hypothermia to be a dose-dependent phenomenon, with patients receiving < 50 mg obtaining excellent protection. Temperature-dependent metabolic processes are required for cellular uptake of doxorubicin (14). In addition, vasoconstriction from hypothermia decreases the amount of drug reaching hair follicles (15). Peak plasma concentrations of doxorubicin during the phase of initial drug distribution are short-lived, with a rapid drop in plasma concentration within the first 15 minutes after iv injection (16). It has been a concern among oncologists that the use of selective hypothermic cooling could lead to ineffective delivery of cytotoxic agents at cutaneous sites which might harbor tumor stem cells, ie, in leukemia and lymphoma. It is our opinion that the use of such a cooling device is contraindicated in solid tumors with known scalp dissemination or in hematologic neoplasms.

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Treatment of Advanced Soft Tissue Sarcomas With Chlorozotocin: A Phase II Trial of the EORTC Soft Tissue and Bone Sarcoma Group[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Chlorozotocin, 120 mg/m² every 4 weeks, was administered to 22 patients with advanced soft tissue sarcoma. All patients had received extensive prior chemotherapy and had measurable progressive disease at the time of entry in the study. No objective responses were observed. The best individual results were in three patients with disease stabilization lasting 2–4 months. Toxic effects included nausea in one half of the patients and sweating episodes in one. One patient developed temporary leukopenia and three had thrombocytopenia that lasted for 3 weeks, 4 weeks, and 4 months. Blood sugar levels were unaffected by the treatment. No renal or hepatic toxicity was recorded.

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Two of the biochemical properties of the nitrosoureas are their abilities to alkylate and to carbamoylate nucleophilic sites on target molecules (1). Chlorozotocin, the 2-chloroethyl analog of streptozocin, has four to five times higher alkylating activity than CCNU and methyl-CCNU but its carbamoylating activity is only about 2% of that of CCNU and methyl-CCNU (2).

Chlorozotocin has shown activity against L1210 and P388 leukemias and against B16 melanoma, with cures being obtained in all three systems.[3] At a 10% lethal dose, chlorozotocin has demonstrated minimal myelosuppression when compared to CCNU and BCNU (3,4).

Three phase I studies have been published (5–7). In one study (5) 43 patients received single iv doses ranging from 5 to 175 mg/m² every 6 weeks. No hematologic toxicity was observed at doses < 120 mg/m² but thrombocytopenia occurred at higher doses. In a second study (6) chlorozotocin was administered to 35 patients using either single-day or 5-consecutive-day schedules repeated every 6 weeks. On either schedule, dose-limiting toxic effects were thrombocytopenia and leukopenia at total doses of 150–200 mg/m². In a third study (6) chlorozotocin was given to 37 patients in a 5-day schedule every 6 weeks. Total dose for each course ranged from 75 to 200 mg/m². Again, myelosuppression consisted of dose-limiting thrombocytopenia occurring regularly at doses of 150 mg/m² and leukopenia occurring only at the highest dose level.

Nausea and vomiting have been mild and infrequent (5–7). Minor reversible elevations of hepatic transaminases have been observed in some cases (5,7). Blood sugar was monitored in one study (6) and was normal in all patients during treatment.

In each of the two phase I studies (5,7), three patients with evaluable advanced soft tissue sarcomas were treated, with no response. In another four patients with advanced sarcomas, two objective responses have been observed.[4,5]

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[2]This study was conducted by the European Organization for Research on Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group at the following institutions: Finsen Institute, Copenhagen, Denmark (*H. T. Mouridsen*); Christie Hospital and Holt Radium Institute, Manchester, England (*V. H. C. Bramwell*); Centro de Oncologia, Hospital General de Asturias, Oviedo, Spain (*J. Lacave*); Centre Alexis Vautrin, Nancy, France (*R. Metz*); Academisch Ziekenhuis, Utrecht, The Netherlands (*C. Vendrik*); Hôpital Louis Pasteur, Colmar Cedex, France (*J. Hild*); and EORTC Data Center, Institut Jules Bordet, Brussels, Belgium (*J. McCreanney* and *R. Sylvester*).

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[3]Helman LJ, Louie A, and Slavik M. Clinical brochure: chlorozotocin (NSC-178248). Prepared by the National Cancer Institute, Bethesda, MD, Aug 1976.

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The objective of this trial conducted by the Soft Tissue and Bone Sarcoma Group of the European Organization for Research on Treatment of Cancer (EORTC) was to study the efficacy and the toxicity of chlorozotocin in patients with advanced soft tissue sarcoma resistant to previous combination chemotherapy. According to the results from the previous phase I studies (6), it was considered feasible to use a dose of 120 mg/m² administered every 4 weeks.

MATERIALS AND METHODS

Patients.—The conditions for patient eligibility were (*a*) advanced measurable soft tissue sarcoma, (*b*) resistance to previous conventional combination chemotherapy, (*c*) age between 15 and 75 years, (*d*) Karnofsky index of ≥ 50, (*e*) normal kidney function, and (*f*) normal hematologic status (ie, wbc count of ≥ 3 × 10^3/μl and platelet count of ≥ 120 × 10^3/μl). Patients with central nervous system lesions, intercurrent infection, overt psychosis, or marked senility were not included in the study.

Investigations.—Pretreatment investigations included clinical evaluation with measurement of lesions, determination of Karnofsky index, cbcs, kidney function tests (serum creatinine or creatinine clearance), liver function tests (serum bilirubin, alkaline phosphatase, SGOT, and SGPT), blood sugar tests, chest roentgenogram, and in some cases bone scan or bone roentgenograms. Cbcs and clinical evaluation were repeated before each course; the other investigations were repeated after two courses, at 8 weeks, and then at 2–3-month intervals.

Treatment.—A single iv dose of chlorozotocin (120 mg/m²) was administered every 4 weeks. The response was evaluated after the second course. If at 8 weeks there was tumor regression or stable disease, treatment was continued until disease progression. If there was disease progression at 8 weeks, the patient was removed from the study.

Criteria of evaluation.—Patients were considered to be evaluable if they received a minimum of two courses of treatment.

RESULTS

Twenty-two patients were entered in the study. Their characteristics are summarized in table 1, and their histologic diagnoses are shown in table 2.

All patients had received extensive prior chemotherapy. This is shown in table 3, which also includes the response to the previous treatment.

There were no objective remissions; 14 patients had progressive disease and three had stabilization or < 50% regression of the disease for 4, 3½, and 2 months. Five patients were not evaluable for response at 8 weeks: one because of death from the malignant disease 11 days after the first course, and four because of rapidly progressing disease with subsequent discontinuation of treatment at 4 weeks.

Patients received one to four courses of chlorozotocin (median, two courses), with doses equivalent to 50%–110% (mean, 96%) of the precalculated dose.

The drug was fairly well-tolerated. Moderate nausea and vomiting occurred in one half of the patients and one patient reported sweating episodes 3–5 days after drug administration. One patient had leukopenia (wbc count, 1.9 × 10^3/μl) that subsided after 1 week. Three patients developed thrombocytopenia after the first course. In one case the platelet count remained between 6.0 and 25.0 × 10^3/μl for 4 months until death. During this period the patient had progression of a soft tissue lesion. Roentgenograms of the skeleton were normal; bone marrow examination was not done. In two cases the platelet count decreased to 13.0 and 58.0 × 10^3/μl 4 weeks after administration of the drug but returned to normal after another 4 and 3 weeks, respectively. Bone marrow examinations demonstrated tumor infiltration in the marrow in the first

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Male	9
Female	13
Age (yrs)	
Median: 48	
Range: 21–72	
Karnofsky index	
Median: 80	
Range: 50–100	
Extent of disease	
Local	3
Distant	13
Local + distant	6

TABLE 2.—Histologic diagnoses

Diagnosis	No. of patients
Angiosarcoma	3
Fibrosarcoma	5
Leiomyosarcoma	4
Liposarcoma	2
Neurofibrosarcoma	2
Rhabdomyosarcoma	2
Miscellaneous	4

TABLE 3.—Prior treatments and results

	No. of patients
Prior treatment*	
CYVADIC	11
CYVADIC→Cis	5
CYVADIC→M→Bl + Dact→Cis	1
CYVADact	1
CYVA	1
CYVA→Q	1
CYVA→Cis	1
A→CYV	1
Results of prior treatment	
Partial or complete response	11
No change	3
Disease progression	6
Not evaluable†	2

*CY = cyclophosphamide, V = vincristine, A = doxorubicin, DIC = DTIC, Cis = cisplatin, M = methotrexate, Bl = bleomycin, Dact = dactinomycin, Q = quelamycin.
†Adjuvant treatment.

of these cases. Among the three patients with thrombocytopenia, two had previously received CYVADIC and one had received doxorubicin followed by vincristine and cyclophosphamide. Thus, as regards prior treatment, these patients did not differ from the other patients. Blood sugar was monitored in 18 of the patients and was normal. There was no renal or hepatic toxicity.

DISCUSSION

In four previous studies, two of ten patients with advanced soft tissue sarcomas responded to chlorozotocin (2,5,6; *see* footnote 3), but the previous treatment given to these patients was not mentioned. However, no responses were observed in the present study including 22 patients, of which 17 received at least two courses of treatment. These poor results do not exclude an activity of chlorozotocin in previously untreated soft tissue sarcomas. However, in view of the 30%–40% response rate of sarcomas to doxorubicin (8) it is difficult to deny patients the chance to receive treatment with doxorubicin before going on to receive investigational agents. The current approach of the EORTC sarcoma group is to evaluate new anthracyclines as single agents and then to go directly to other investigational agents. This is possibly one setting in which a future evaluation of chlorozotocin or other new drugs might be useful. Another might be in randomized trials to compare the efficacy of active anthracyclines or combinations with and without simultaneous chlorozotocin.

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Chlorozotocin: Phase II Evaluation in Patients With Advanced Sarcomas[1,2,3]

Brief Reports and Preliminary Communications

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Chlorozotocin is a water-soluble nitrosourea with high alkyating and low carbamoylating activities (1); it has demonstrated activity in several animal tumor systems including B16 melanoma and L1210 leukemia. Occasional responses have been noted in patients with lymphoma (2), Hodgkin's disease (3), melanoma (2,4), and carcinomas of the colon (4), breast (4), and ovary (5). Published phase II studies of chlorozotocin used against carcinomas of the lung (6,7) and kidney (8) have shown minimal activity. A phase II trial of this agent was undertaken to assess its activity against soft tissue sarcomas.

MATERIALS AND METHODS

Twenty-seven patients with histologically confirmed sarcomas and objectively measurable disease were entered in this study. All patients had received prior chemotherapy, although no patient had received a nitrosourea. In addition, ten patients had received radiotherapy. The histologic types of these patients were as follows: eight with leiomyosarcoma, five with fibrosarcoma, three with liposarcoma, three with synovial sarcoma, two each with malignant fibrous histiocytoma and hemangiopericytoma, and four with miscellaneous sarcomas.

There were 15 males and 12 females. The median age was 51 years (range, 20–74), and the median performance status on the Karnofsky scale was 70 (range, 50–90). Sixteen patients had pulmonary metastases, 12 had liver metastases, seven had subcutaneous metastases, and one had brain metastases.

Patients were considered evaluable 6 weeks after the start of chlorozotocin therapy. Therapeutic response was defined as follows: *partial remission (PR)*—a $\geq 50\%$ decrease of all measurable tumor masses for > 1 month; and *minor response (MR)*—an objective tumor decrease $> 25\%$ but less than that required for a PR.

Chlorozotocin was administered as a rapid iv injection in equally divided doses for 3 consecutive days. The initial total dose for patients with a performance status ≥ 70 was 180 mg/m^2. Patients with a performance status of 50 or 60 received an initial total dose of 150 mg/m^2. Courses were repeated every 6 weeks if the platelet count had returned to $\geq 100{,}000$/mm^3 and if the wbc count had returned to ≥ 3500/mm^3. Chemotherapy was delayed 1 week if the platelet or wbc count was less than these levels. If no myelosuppression was observed, the subsequent course was given at a total dose 30 mg/m^2 higher than the previous course. If myelosuppression was observed with a platelet count $< 50{,}000$/mm^3 or a wbc count < 2000/mm^3, the next total dose of chlorozotocin was reduced by 30 mg/m^2. In five patients, the highest dose received was 240 mg/m^2; in nine other patients, the highest dose was 210 mg/m^2. Seven patients received maximum doses of 180 mg/m^2, while six patients were given 150 mg/m^2.

RESULTS

Therapeutic Responses

All patients received an adequate trial of chlorozotocin. No PRs occurred. MRs were noted in two patients. One occurred in a 70-year-old woman with multiple subcutaneous metastases from a low-grade fibrosarcoma. The other MR consisted of a decrease in a malignant pleural effusion in a patient with metastatic malignant schwannoma. Both of these responses were brief, lasting 2 and 3 months, respectively.

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[3]Chlorozotocin was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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TABLE 1.—Hematologic toxicity with chlorozotocin

Wbc count nadir (cells/mm³)	No. of patients	Platelet count nadir (cells/mm³)	No. of patients	Hemoglobin decrease (g/100 ml)	No. of patients
≥ 4000	10	≥ 150,000	4	0–1.9	10
3000–3900	11	100,000–149,000	7	2.0–2.9	8
2000–2900	4	50,000–99,000	11	3.0–3.9	5
1000–1900	2	< 50,000	5	> 4.0	4
< 1000	0				

Toxicity

The 27 patients received a median of two courses of chlorozotocin (range, one to four). Hematologic toxicity was the most frequent dose-limiting factor (table 1). The median platelet count nadir of the 27 patients was 80,000 cells/mm³ (range, 15,000–301,000). The median wbc count nadir was 3600 cells/mm³ (range, 1900–6400).

One patient required platelet transfusions as the result of chlorozotocin-induced thrombocytopenia, but there were no episodes of severe hemorrhage secondary to chemotherapy. One patient with fibrosarcoma metastatic to the lungs developed shortness of breath and a bilateral interstitial infiltrate visible in a chest roentgenogram after the third course of chlorozotocin, in which he received 240 mg/m². Pulmonary function tests revealed a moderate to severe decrease in diffusion capacity. Chemotherapy was discontinued, and after 6 weeks, the symptoms and chest roentgenogram improved without specific therapy. The patient received chlorozotocin again after this episode, without recurrence of pulmonary symptoms. No evidence of hepatic or renal toxicity occurred in any of the 27 patients. Some patients experienced mild nausea and vomiting.

DISCUSSION

Twenty-seven patients with soft tissue sarcomas were treated with chlorozotocin. The drug was well-tolerated; thrombocytopenia was the most prominent toxic effect. One patient developed an interstitial pneumonitis similar to that described with other nitrosoureas (9–11).

No major responses occurred with chlorozotocin among these 27 patients. Although all patients entered in this study had received prior chemotherapy, the median performance status of the group was good, and patients received a median of two 6-week courses of treatment. It may be concluded that chlorozotocin at this dose and schedule has a predicted true major response rate of ≤ 11% ($P = 0.05$) and a predicted true MR rate of < 23% ($P = 0.05$) in this patient population. This drug appears to have little therapeutic value as a single agent in previously treated patients with sarcomas.

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Phase II Evaluation of Vindesine Sulfate in Patients With Advanced Sarcomas[1,2,3]

Brief Reports and Preliminary Communications

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Vindesine is a new agent of the vinca alkaloid class derived from vinblastine. Clinical trials have used vindesine on a schedule of 3–4 mg/m²/week (1,2), with objective responses seen against carcinomas of the lung (3), breast (4), and testis (5) as well as lymphomas and leukemias (6,7). No activity has been seen using this dose against renal cell carcinoma (8). Because of the reported activity of older vinca alkaloids against soft tissue sarcomas (9) and because responses have been noted with vindesine used against human sarcomas heterotransplanted in nude mice (10), a phase II study of vindesine was undertaken to assess its activity against this tumor.

MATERIALS AND METHODS

Fifty-four patients with histologically confirmed sarcomas and objectively measurable disease were entered in this study. Forty-one patients had received vincristine and at least one prior multiple-drug regimen. Thirteen patients had received no prior chemotherapy, and 31 patients had received prior radiotherapy. The histologic types of these patients were as follows: 15 with leiomyosarcoma; nine with liposarcoma; five with fibrosarcoma; four with malignant fibrous histiocytoma; three with synovial sarcoma; two each with hemangiopericytoma, giant cell sarcoma, alveolar soft tissue sarcoma, chondrosarcoma, lymphangiosarcoma, embryonal rhabdomyosarcoma, and mesothelioma; and four with miscellaneous sarcomas. There were 24 males and 30 females. Median age was 51 years (range, 20–80), and median performance status on the Karnofsky scale was 80 (range, 50–90). Thirty-nine patients had pulmonary metastases, 11 had subcutaneous metastases, eight had liver metastases, and two each had metastases to bone and brain.

An adequate trial required at least three doses of vindesine accompanied by dose-limiting hematologic or neurologic toxicity. Therapeutic responses were defined as follows: *complete remission*—the disappearance of all evidence of disease for > 1 month; *partial remission (PR)*—a ≥ 50% decrease of all measurable tumor masses for > 1 month; and *minor response (MR)*—objective tumor decrease > 25% but less than that required for a PR. Vindesine was administered weekly by rapid iv injection. The initial dose was 3 mg/m²; if no toxicity occurred, this was escalated by 0.5-mg/m² increments to a maximum of 5 mg/m². The dose was reduced by 50% if the wbc count was 2000–2900/mm³ or if the platelet count was 75,000—99,000/mm³. The drug was withheld for 1 week if the wbc or platelet count was less than these levels or if functional neuropathy developed. Patients who continued on chemotherapy for more than six doses were treated every 2 weeks instead of weekly. Informed consent was obtained from all patients before beginning therapy.

RESULTS

Fifty-four patients were entered in this study. Eight patients discontinued therapy or died of their disease after receiving one or two doses of vindesine, and their trials were judged to be inadequate.

The toxic effects of vindesine were mostly hematologic or neurologic. Depression of the wbc count occurred in most patients (nadir range, 1000–6500/mm³; median, 2800). Serious depression of the platelet count was not seen (nadir range, 103,000–328,000/mm³; median, 234,000). Thirty-seven patients developed neuropathy. This usually consisted of numbness and paresthesias. In two patients who each received 14 doses of vindesine, the neuropathy was severe enough to cause difficulty in walking. Symptoms usually resolved within

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[3]Vindesine was supplied by Eli Lilly and Co, Indianapolis, IN.

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[5]We thank Orlando Hernandez for aid in the analysis of these data.

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weeks after withholding therapy, although in these two patients, paresthesias continued for several months after the last dose of vindesine. Constipation occurred in approximately one half of the patients. Thirteen patients complained of severe malaise and myalgias up to a week after the dose of vindesine, and 11 had fevers to 103°C for several days after chemotherapy. Some patients experienced severe headache or jaw pain. No patient had hepatic or renal toxicity. Alopecia was significant in 29 patients. Phlebitis and local ulceration at the injection site were not noted in any of the patients.

There was no apparent difference in the incidence of hematologic toxicity or neurotoxicity between patients who had had prior vincristine therapy and those who had not, nor did the incidence of neurotoxicity in these patients appear to be related to age.

The 46 adequately treated patients received a median of six doses of vindesine (range, three to 27). In the 33 patients who were previously treated, there were one PR and four MRs. Four patients were believed to have stable disease. The PR consisted of a 90% decrease in the size of a large abdominal liposarcoma in a 72-year-old woman. This response lasted 8 months. The four MRs lasted 1, 2, 2, and 3 months.

In the 13 patients who were not previously treated with chemotherapy, there were two PRs and four MRs. One PR (3 months) consisted of a 60%–70% decrease in multiple pulmonary metastases in a man with giant cell sarcoma. The other PR (2 months) occurred in a 41-year-old woman with lymphangiosarcoma and consisted of a 70% decrease in multiple subcutaneous metastases. The MRs lasted 1, 2, 2, and 3+ months. Three patients have had stable disease, and four have had disease progression while receiving therapy. All responses in both groups of patients were apparent within 4 weeks of therapy.

DISCUSSION

Forty-six patients with soft tissue or bone sarcomas were adequately treated with vindesine. The drug was well-tolerated; leukopenia, neuropathy, and alopecia were the most prominent toxic effects. Malaise, myalgias, and fever were also frequently experienced.

One PR (3%) and four MRs (12%) were seen in 33 patients previously treated with chemotherapy, while two PRs (15%) and four MRs (31%) were seen in 13 previously untreated patients. On this basis, for the population treated it can be predicted that vindesine has a true PR rate of 2%–17% and a true PR plus MR rate of 13%–40% ($P = 0.05$). Previous studies with vincristine have shown a 14% observed PR rate against sarcomas (9); however, the minimum criteria of PR in these studies was similar to our criteria for MR.

Previous studies with vindesine against breast carcinoma (4) and lung carcinoma (3) have shown higher response rates in previously untreated patients than in previously treated patients. With some evidence of activity in patients with this relatively chemoresistant disease, it would appear that further trials of vindesine are indicated in patients who have not had prior chemotherapy.

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Phase II Study of the Combination of Dianhydrogalactitol, Doxorubicin, and Cisplatin (DAP) in Patients With Advanced Squamous Cell Lung Cancer[1,2]

Brief Reports and Preliminary Communications

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In 1977 (1), we reported on our experience with dianhydrogalactitol (DAG) and the combination of cyclophosphamide, doxorubicin, and cisplatin (CAP). In patients with squamous cell lung cancer (SQCLC) and no prior chemotherapy, we observed a 15% (four of 26 patients) regression rate for DAG and a 44% (eight of 18 patients) regression rate for CAP. Since the 15% regression rate for DAG was superior to the 4% regression rate noted with cyclophosphamide (2), we did a subsequent randomized study in a similar population of patients with SQCLC (3), comparing DAG and doxorubicin versus DAG, doxorubicin, and cisplatin (DAP). The three-drug regimen was superior to the two-drug regimen in regression rate (53% vs 27%), median regression duration (175 vs 58 days), and median survival time (185 vs 126 days). Since the doses of DAG and doxorubicin were identical in both regimens, we believed that cisplatin was probably playing a significant role. The dose of cisplatin in both the original CAP study (1) and in the DAP regimen (3) was 40 mg/m^2. We wanted to see whether or not increasing the cisplatin dose by 50% to 60 mg/m^2 would be tolerable and whether or not it would improve the efficacy of the DAP regimen. If the benefit from cisplatin was secondary to drug synergism rather than being due to individual drug antitumor effect, the increase in drug dose might be of minimal or no additional clinical benefit if the 40 mg/m^2 was optimal or adequate for achieving the possible synergistic effect. Consequently, this phase II study of the new DAP combination with a higher cisplatin dose was conducted.

MATERIALS AND METHODS

To be eligible for the study, patients were required to have the following: histologic or cytologic diagnosis of SQCLC, measurable or evaluable disease, normal wbc and platelet counts, a serum creatinine < 1.5 mg/dl, no active significant cardiac disease, an Eastern Cooperative Oncology Group Performance Score (ECOG PS) ≤ 3, no prior chemotherapy, and a signed informed consent. To be considered evaluable, patients had to have met the above eligibility criteria and have completed one full 3-day course of chemotherapy.

Forty patients were entered in the study and three (7.5%) were disqualified: one with no measurable or evaluable disease, one who had an ECOG PS of 1 when entered in the study but an ECOG PS of 4 (marasmic endocarditis with widespread emboli and disseminated intravascular coagulation) when treatment was begun 1 day later, and one who withdrew from the study because of subjective toxicity after the first day of treatment (a complete regression [CR] was noted on evaluation 30 days later). Of the 37 evaluable patients, 28 had both an untreated primary tumor and distant metastases, while seven had prior thoracic irradiation and two had prior resection. Thirty-six of the 37 evaluable patients were male, and the median age of the group was 63 years (range, 43–75). Twenty-six patients had an ECOG PS of 0–1 and 11 had an ECOG PS of 2–3. The indicator lesion (usually lung primary) was evaluable in 25 patients and measurable in 12.

Separate response criteria were used for measurable and evaluable (clearly visible but not clearly measurable) disease. These have been reported in detail elsewhere (4). A partial regression of measurable disease ($\geq$ 50% reduction in the product of perpendicular diameters) was equivalent to a regression (definite decrease in tumor size agreed upon by two observers) of evaluable disease. Patients were taken out of the study at the first sign of tumor progression. Only the first CNS disease

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progression was not considered progression. Patients with CNS metastases at time of entry in the study received whole-brain radiation therapy immediately.

Chemotherapy consisted of DAG, 25 mg/m²/day for 3 consecutive days by rapid iv infusion; doxorubicin, 40 mg/m² on Day 1 only by rapid iv infusion; and cisplatin, 20 mg/m²/day for 3 consecutive days given as a 1-hour infusion in 500 ml of 5% dextrose in one half normal saline without mannitol or diuretics. Cisplatin doses were reduced by 25% for serum creatinine levels of 1.3–1.4 mg/dl and by 50% for serum creatinine levels of 1.5–1.9 mg/dl. Cisplatin was permanently discontinued if serum creatinine increased to ≥ 2.0 mg/dl.

RESULTS

Tumor Regression

Overall, 20 of 37 (54%) patients had tumor regressions, seven of 12 (one CR and six partial regressions) with measurable disease versus 13 of 25 (including one CR) with evaluable disease. ECOG PS did not seem to affect regression of tumor, with a 50% regression rate noted for patients with an ECOG PS of 0–1 versus a 64% regression rate for patients with an ECOG PS of 2–3. There was a suggestion in our earlier study of DAP (3) that older patients responded better, but we could detect no difference in regression rates for patients < 63 years of age (ten of 18; 56%) versus those ≥ 63 years old (ten of 19; 54%) in this study.

Time to Tumor Progression

The median time to tumor progression was 113 days (range, 8–388) for all patients and ranged from a median of 53 days for patients with no regression to 169 days for patients with tumor regressions. Age (< 63 vs ≥ 63 years) did not affect this parameter with median times of 93 and 113 days, respectively.

Survival

Overall survival time for all 37 evaluable patients was 229 days (range, 8–388+). Patients with tumor regressions survived significantly longer ($P = 0.0005$), with a median of 378 versus 171 days for patients with no tumor regression. This could not be explained by ECOG PS differences alone since the regression rate was higher (64% vs 50%) in the patients with poorer scores (ECOG PS 2–3 group).

Time to tumor progression and survival time were calculated from Kaplan-Meier plots (5) and analyzed using Gehan-Wilcoxon test statistics (6).

Toxicity

Nausea and vomiting were moderate to severe in almost all patients, being most marked on Day 1 and decreasing in intensity over the next 2 days of treatment. Of the 37 evaluable patients, two refused further treatment after three and four courses of DAP because of this type of toxicity.

There were ten patients (27%) who developed increases in serum creatinine above normal or baseline. No elevation was > 1.9 mg/dl. There was no apparent age correlation.

Hematologic toxicity was significant. Most patients receiving more than one course of therapy experienced 2–4-g/dl decreases in their hemoglobin levels. No proven episodes of hemolysis were documented.

Interval wbc and platelet toxicity data were available on 29 patients after the first course of therapy. Leukopenia (wbc count < 4 × 10³/mm³) and thrombocytopenia (platelet count < 130 × 10³/mm³) occurred in 93% and 83% of the cases, respectively. Nine episodes of leukopenia were < 1.5 × 10³/mm³ and eight episodes of thrombocytopenia were < 50 × 10³/mm³. Considering all cycles of treatment, all but one patient developed leukopenia (median nadir, 2100 cells/mm³; range, 700–3800 cells/mm³) and thrombocytopenia (median nadir, 56,000 cells/mm³; range, 3000–129,000 cells/mm³). Despite significant hematologic toxicity, there were no drug-related deaths.

All patients developed alopecia. One patient had congestive heart failure after receiving a total doxorubicin dose of 80 mg/m².

DISCUSSION

The 54% regression rate noted in 37 patients in this study corroborates the 53% regression rate noted in 19 patients with SQCLC reported earlier (3). The 50% increase in cisplatin from 40 mg/m² given on 1 day in the earlier study to 20 mg/m²/day for 3 days (60 mg/m² total) in this study did not seem to greatly influence the efficacy of the DAP regimen, although this point was not evaluated in a prospective randomized fashion. The regimen with the higher dose of cisplatin resulted in a shorter median time to tumor progression (113 vs 175 days) and regression duration (169 vs 255 days) but a better median survival time (229 vs 185 days).

There was no major difference in the efficacy of the subsequent therapy offered patients in both studies that would account for the longer survival from tumor progression to death in this study. The populations were quite comparable in sex ratio, ECOG PS, and prior treatment, although the patients in the current study were slightly older (median age, 63 vs 57 years).

The survival advantage for older patients noted in our earlier report (3) could not be confirmed in this study. The previous data probably did reflect a random sampling error as originally suspected.

The current DAP regimen (cisplatin, 60 mg/m^2) is very myelosuppressive, probably reflecting an interaction of DAG and cisplatin. The platelet toxicity was especially worrisome. Unless DAP could be shown to be therapeutically superior to CAP, we feel that the more frequent and severe myelosuppression from DAP makes it a less attractive therapy in advanced disease and too potentially dangerous for adjuvant use.

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Phase I–II Study of 3-Deazauridine in Adults With Acute Leukemia[1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

3-Deazauridine, a uridine analog, was administered to 36 patients with acute leukemia by both intermittent and continuous iv infusion at doses ranging from 400 to 6000 mg/m²/day × 5, repeated at 1–3-week intervals. There were no complete or partial responses but three patients showed hematologic improvement. Major toxic effects were myelosuppression, stomatitis, vomiting, diarrhea, and skin erythema. Daily doses > 3000 mg/m² were associated with significantly increased toxicity, including three drug-related deaths. The recommended dose for future studies is 2500 mg/m²/day as a continuous 5-day infusion.

[Cancer Treat Rep 65:521–524, 1981]

3-Deazauridine is a structural analog of uridine that is marginally active against murine L1210 leukemia but highly active against L1210 leukemia resistant to cytarabine (1). It is metabolized in the cell to deazauridine triphosphate and is thought to act by interfering with DNA synthesis by blocking cytidine triphosphate synthetase activity (1,2). The drug was not active against P388 leukemia, B16 melanoma, or Lewis lung carcinoma. In preclinical toxicology studies in large animals, toxic effects included emesis, bloody diarrhea, anorexia, skin ulcerations and necrosis, renal and hepatic dysfunction, lymphoid depletion, and bone marrow hypoplasia.[5]

This study was designed to determine the maximum tolerated dose and the efficacy of 3-deazauridine when used alone in previously treated patients with acute leukemia. Preclinical investigations revealed little schedule dependency with 3-deazauridine; we therefore investigated the drug when given in single injections daily for 5 days, repeated after bone marrow recovery. Subsequently, the schedule was changed to a 5-day continuous infusion when pharmacologic studies conducted by Benvenuto et al (3) became available, showing increased plasma half-life and extrapolated volume of distribution with continuous infusion of the drug. The toxic dose low for both dogs and monkeys was 1250 mg/m²/day × 5. Hence, one third of this dose was approximately 400 mg/m², which was chosen as the starting dose.

MATERIALS AND METHODS

Thirty-six previously treated patients with acute leukemia were entered in this study. There were 22 men and 14 women with a median age of 31 years (range, 17–75). Thirty patients had acute myelogenous leukemia, four had acute lymphoblastic leukemia, one had chronic myelogenous leukemia in blast cell crisis, and one had lymphoma/leukemia. All patients had had extensive prior therapy including cytarabine given either in combination or sequentially.

The initial starting dose of 3-deazauridine was 400 mg/m²/day × 5 given by intermittent rapid iv infusion. Doses were escalated to a maximum of 6000 mg/m²/day × 5 and were subsequently reduced to 3000 mg/m²/day × 5 given as a continuous infusion when pharmacokinetic data became available. Courses of treatment were repeated every 1–3

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TABLE 1.—Response to 3-deazauridine*

Dose (mg/m²/day × 5)	No. of courses	No. of courses with reduction of wbcs and/or blast cells	No. of patients with bone marrow hypoplasia
Intermittent schedule			
400–800	8 (3 ALL)	0	0
1600–2000	5 (1 ALL)	2	0
2500	3 (1 ALL)	0	0
3000	2 (1 ALL)	2 (1 ALL)	1
3500	2	0	0
4500–5000	8 (1 ALL, 1 LL, 1 CML)	4	0
6000	1	1	0
Continuous schedule			
2500	13	11	1
3000	26 (1 ALL, 1 LL, 1 CML)	22 (1 ALL)	7 (1 ALL)
3500	2	2	0
6000	1	1	1

*All patients had a diagnosis of acute myelogenous leukemia, except where indicated in parentheses by ALL (acute lymphoblastic leukemia), LL (leukemia/lymphoma), or CML (chronic myelogenous leukemia in blast cell crisis).

weeks provided the patients had recovered from prior drug-related toxicity. Response to treatment was assessed using standard criteria as previously described (4).

RESULTS

Thirty-six adults with acute leukemia were given 71 courses of 3-deazauridine: 29 courses (in 11 patients) as an intermittent infusion and 42 courses (in 28 patients) as a continuous infusion (three patients received intermittent infusion followed by continuous infusion). Hints of antileukemic activity in terms of reduction in the total wbc count and/or peripheral blast cell counts were noted by the second dose escalation (1600 mg/m²/day × 5) (table 1). Since only four patients received doses below this level, all patients were considered evaluable for response. There were no complete or partial responses, but in most patients, there was significant reduction in the total wbc count with either complete or partial clearing of blast cells from the peripheral blood (table 1). In patients who received intermittent therapy, doses ≥ 1600 mg/m² (nine of 21 courses) were associated with reduction in the total wbc count and some clearing of blast cells from the peripheral blood. One patient who received a dose of 3000 mg/m²/day had severe bone marrow hypoplasia. In the patients who received continuous infusion therapy, 36 of 42 courses were associated with clearing of blast cells from the peripheral blood, with nine patients having severe bone marrow hypoplasia. However, in all these patients the bone marrow was rapidly repopulated with leukemic cells within a median time of 2 weeks. Three patients who received continuous 3-deazauridine therapy at doses ≥ 3000 mg/m² had hematologic improvement with clearance of peripheral blast cells and some effect on the bone marrow. In the first patient, the bone marrow blast cell count was reduced to < 5% and was associated with marked reduction in leukemic skin infiltrations, but the patient's peripheral blood counts never returned to normal. In another patient, the bone marrow blast cell count was reduced from 69% to 5.6% but her platelet count never returned to normal. The third patient had complete clearing of his peripheral blast cells and a > 50% reduction in bone marrow blast cells to < 30%.

Toxicity

The number of courses at each dose level and the resultant median lowest blood counts are shown in table 2. Myelosuppression with continuous infusion therapy appeared to be greater than that observed in comparable doses of intermittent therapy. The lowest observed wbc and platelet counts were noted at a median of 11 days for continuous therapy and 6 days for intermittent therapy. In patients who received doses > 2500 mg/m², the incidence of severe bone marrow hypoplasia was 6% (one of 16 patients) with intermittent therapy and 21% (nine of 42) with continuous therapy.

The nonhematologic toxicity associated with 3-deazauridine chemotherapy is shown in table 3. No toxic effects were observed with doses < 1600 mg/m². Dose-limiting toxic reactions encountered in our

TABLE 2.—Hematologic toxicity

Dose (mg/m²/day × 5)	No. of courses	Lowest wbc count (cells × 10^3/μl)			Lowest platelet count (cells × 10^3/μl)		
		Median	Range	Day	Median	Range	Day
Intermittent schedule							
400–800	8	3.0	0.4–43.3	3	10	1–193	5
1600–2000	5	5.9	0.4–17.1	5	87	14–124	5
2500	3	2.4	0.5–56	3	127	6–140	6
3000	2	0.3/5.5	—	9	13/114	—	6
3500	2	0.6/29	—	7	13/108	—	6
4500–5000	8	3.2	0.7–19	6	13	3–65	9
6000	1	0.5	—	7	21	—	9
Continuous schedule							
2500	13	0.9	0.3– 1.9	10	6	1.0–37	10
3000	26	1.1	0.2–30.4	9	12	3–69	9
3500	2	0.3/0.4	—	13	7/9	—	13
6000	1	0.3	—	12	13	—	13

patient population were severe stomatitis and skin desquamation. In patients who received intermittent therapy, doses ≤ 3000 mg/m² were well-tolerated. In those who received doses ≥ 3500 mg/m², stomatitis was observed in four (36%), was severe in three, and was associated with massive swelling of the tongue in one. For patients on continuous therapy, toxic effects at the 2500-mg/m² dose level were generally well-tolerated and only one patient had severe stomatitis. However, at doses ≥ 3000 mg/m², stomatitis was severe in nine patients (31%) and was associated with severe diarrhea in three and generalized erythema and skin desquamation in two.

There were three drug-related deaths, all occurring in patients who received continuous therapy, one each at 3000, 3500, and 6000 mg/m². All three patients had a combination of severe diarrhea, stomatitis, septicemia, and gastrointestinal ulcerations and necrosis at autopsy. Two patients had generalized erythematous rash followed by severe skin desquamation.

DISCUSSION

3-Deazauridine, a uridine analog, was brought to clinical trials because it demonstrated significant antitumor activity against L1210 leukemia resistant to cytarabine. Unfortunately, in this study, we were unable to obtain any significant responses. In our earlier studies with phase I–II agents in previously treated patients with acute leukemia, the complete remission rates have varied from 5% to 33% (4–6). Therefore, even taking into account the advanced stage of disease and degree of prior therapy, it ap-

TABLE 3.—Nonhematologic toxicity

Dose (mg/m²/day × 5)	No. of courses	No. of patients with—							
		Nausea and vomiting	Diarrhea	Stomatitis (severe)	Fatigue	Anorexia	Abdominal pain	Rash	Generalized erythema and skin desquamation
Intermittent schedule									
1600–2000	5	3	0	1	2	0	0	0	0
2500	3	1	1	0	0	1	0	0	0
3000	2	1	1	1	1	1	0	0	0
3500	2	1	2	0	0	0	0	0	0
4500–5000	8	3	1	3 (2)	0	2	0	0	0
6000	1	0	0	1 (1)	0	0	0	0	0
Continuous schedule									
2500	13	2	2	3 (1)	0	0	1	2	0
3000	26	3	1	9 (7)	0	0	1	5	0
3500	2	0	1	1 (1)	0	0	1	0	1
6000	1	0	1	1 (1)	0	0	0	0	1

pears that 3-deazauridine as a single agent has a limited role in the treatment of patients with acute leukemia.

The toxic effects observed in this study were considerable, particularly at doses $\geq$ 3000 mg/m^2. In patients who received continuous therapy, the toxic effects have been lessened by a reduction in the starting dose from 3000 to 2500 mg/m^2. Possibly by changing to the intermittent infusion schedule, higher doses of 3-deazauridine may be better tolerated. We currently recommend a starting dose of 2500 mg/m^2/day by 5-day continuous infusion.

It is unlikely that 3-deazauridine will prove to be useful in the treatment of patients with acute leukemia when used alone, since its therapeutic dose is higher than its maximally tolerated dose. However, at lower doses, it may well have a role in combination chemotherapeutic regimens where it may potentiate the effect of other drugs, for example, cytarabine, by lowering intracellular cytidine triphosphate levels (7).

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Phase II Trial of Methyl-GAG in Advanced Renal Cancer[1,2]

Brief Reports and Preliminary Communications

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Methyl-GAG (M-GAG), a drug introduced in the early 1960s (1), is being re-evaluated using a new weekly iv schedule (2–7). Compared to the older daily dose schedules, weekly administration has produced fewer toxic effects, ie, mucositis, nausea and vomiting, and myelosuppression (2). Since preliminary reports (2–5) have described a few responses in patients with metastatic hypernephroma, a phase II disease-oriented trial of M-GAG was undertaken in patients with bidimensionally measurable renal cancer at the Memorial Sloan-Kettering Cancer Center.

MATERIALS AND METHODS

At the Memorial Sloan-Kettering Cancer Center, all patients had a complete history, physical examination, pathologic confirmation of renal cancer, automated blood cell and platelet counts, 12-channel biochemical screening profile, and chest roentgenograms. Radionuclide liver and bone scans, iv pyelograms, a 24-hour urinary creatinine clearance, and a computerized transaxial tomogram (CT scan) were obtained when indicated. Perpendicular diameters of each accessible lesion were measured separately by two investigators and all radiologic studies were evaluated independently. Initially, tumor measurements during physical examination and blood cell counts were performed weekly; screening profiles, serum creatinine, and chest roentgenograms were performed monthly; and radionuclide and CT scans were performed every 6–8 weeks.

Patient selection was strict and required the presence of objectively measurable, bidimensional lesions such as lymphadenopathy, subcutaneous or cutaneous tumors, peripheral pulmonary lesions, malignant hepatomegaly evaluable by physical examination and radionuclide liver and/or CT scans, and a biopsy-proven intra-abdominal or pelvic tumor measurable by CT scan. Other criteria for patient entry included a Karnofsky performance score (PS) > 50%, a life expectancy > 2 months, a wbc count > 3000/mm^3, a platelet count > 100,000/mm^3, and patient consent.

Response criteria included: *complete remission (CR)*—the disappearance of all evidence of disease for > 1 month; *partial remission (PR)*—a ≥ 50% decrease in the sum of the products of the perpendicular diameters of all lesions and a ≥ 50% decrease in all abnormally elevated biochemical parameters; *minor response*—a 25%–50% objective decrease of all measurable parameters; *disease stabilization*—a < 25% change (increase/decrease) for ≥ 3 months; and *disease progression*—a > 25% increase in measurable disease or a mixed response. Durations of response and survival were measured from the start of therapy. An adequate trial of M-GAG required a minimum of three consecutive doses with toxicity and a 1-month survival. PS was used to evaluate subjective response.

The initial dose of M-GAG was 600 mg/m^2 iv every week for good-risk patients (PS > 70%, radiation therapy to limited bone marrow-containing areas, and less than two drug trials) and 500 mg/m^2 iv every week for poor-risk patients. However, the rapid development of fatigue, lethargy, mucositis, pain on swallowing, retrosternal burning, and pharyngitis resulted in de-escalation of the initial doses to 500 and 400 mg/m^2, respectively. M-GAG was diluted in 5% dextrose in normal saline and infused over a 60–90-minute period; rapid infusion produced mild perioral numbness and tingling, headache, and sensation of body warmth.

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PATIENT CHARACTERISTICS

Thirty-one patients with renal cell carcinoma (hypernephroma) and two with adult Wilms' tumor received M-GAG. Patient characteristics are shown in table 1. Nine patients had a PS of 90%–100%, 15 had a PS of 70%–80%, eight had a PS of 60%, and one had a PS of 50%. Excluding the two patients who had adult Wilms' tumor, 70% (21 of 31 patients) had received no prior irradiation, 60% (19 patients) had received no prior chemotherapy, and 73% (23 patients) had had no prior hormonal therapy. Fifteen of 31 patients (48%) had been treated with a hormone or an antineoplastic drug, but none had achieved a clinically significant response (CR or PR) with such therapy. Nine patients with hypernephroma had an abnormally elevated serum calcium (> 10.8 mg/100 ml), and 19 adequately treated patients (61%) had an abnormally elevated serum BUN (> 20 mg/100 ml) or creatinine (> 1.4 mg/100 ml). Two of seven patients had an elevated CEA (> 5.0 mg/100 ml). Thus far, 14 patients (42%) have died.

TABLE 1.—Patient characteristics

Characteristic*	
Total No. of patients	33
Males	79%
Age in yrs	
Median	61
Range	20–71
PS	
Median	70%
Range	50%–100%
Prior therapy	
Nephrectomy	70%
Irradiation	33%
Chemotherapy	42%
Hormonal therapy	24%
Indicator lesions	
Lung	24 (24)†
Lymph nodes/subcutaneous	2 (3)
Abdomen (computerized transaxial tomogram)	5 (8)
Liver	2 (8)
History (mos)	
Symptoms to diagnosis	
Median	2.5
Range	0.2–24
Diagnosis to metastases	
Median	1
Range	6–48
Diagnosis to protocol	
Median	13
Range	0.2–624
Protocol to followup or death	
Median	4+
Range	1–8+
Died	42%

*Unless otherwise specified, values = No. of patients.
†Values in parentheses = total No. of measurable lesions.

RESULTS

Thirty-one of 33 patients had an adequate trial. No patient achieved a PR or a CR. One patient had disease stabilization for 3 months, one had a mixed response (some pulmonary nodules increased while others decreased) for 6 weeks, and three had minor remission for 4–6 weeks.

Two inadequately treated patients, both with a PS of 60%, received only one dose. In 31 adequately treated patients, the mean and median number of doses were 5.9 and 5.0, respectively (range, 3–14+ doses). The initial dose was 600 mg/m² in 13 patients, 500 mg/m² in eight, and 400 mg/m² in eight. One patient with abnormal renal function received an initial dose of 200 mg/m² as part of a pharmacokinetic study and thereafter seven doses of 400 mg/m². Pharyngitis, mucositis, and, particularly, generalized lethargy, weakness, and myalgia were dose-limiting and resulted in dose modification and interruption of the planned weekly schedule. At the 600-mg/m² dose level, four patients received four to six consecutive weekly doses, three received three weekly doses, and six received two weekly doses. Doses were delayed 1–3 weeks and thereafter were continued weekly or every other week. At this level, one patient had the dose increased (700 mg/m²) and three had the dose decreased (400–500 mg/m²). At the 500-mg/m² dose level, four patients received four consecutive weekly doses, two received three weekly doses, and three received two weekly doses. At this level, two had the doses increased (600 mg/m²) and two had the doses decreased (400 mg/m²). At the 400-mg/m² dose level, three patients received four to six consecutive weekly doses, two received three weekly doses, and three received two weekly doses. At this level, four patients had the dose increased (500–600 mg/m²) and one had the dose decreased (250 mg/m²).

While myelosuppression was unusual (< 5%), generalized weakness, fatigue, and lethargy occurred in 63% and disabling myopathy, primarily of the lower extremities, occurred in 17%. Four patients required hospitalization and three additional patients required emergency room treatment. In two patients, serum aldolase and creatine phosphokinase levels and electromyography were normal. After interrupting therapy for 1–3 weeks, similar symptoms recurred rapidly, generally within one to two doses, and thus suggested a cumulative

drug effect which was somewhat dependent on the level of renal function. Other toxic effects included mucositis, pharyngitis, and esophagitis (37%) with retrosternal burning and dysphagia, anorexia, nausea, and, rarely, vomiting (27%), a diffuse papular rash (10%), diarrhea (20%), headache (7%), and nasal stuffiness with temporary blurring of vision (3%).

DISCUSSION

Initially, Knight et al (2) reported one response (CR or PR) of 5 months' duration and two mixed responses in five adequately treated (more than two doses at $\geq$ 500 mg/m^2 every week) patients with hypernephroma. A recent update of the Southwest Oncology Group (3) described one CR, two PRs, and one mixed response in 14 previously untreated patients given M-GAG at a starting dose of 500 mg/m^2, with a dose escalation of 100 mg/m^2. An additional three patients had disease stabilization, and among nine previously treated patients, three had a mixed response. Generally, one half of the responses occurred at three doses or less and one half occurred at four doses or more. Toxic effects included mucositis (43%), nausea and vomiting (26%), diarrhea (9%), and myelosuppression (8%–13%). Using a similar dose schedule, Killen et al (4) noted one response of > 1 month's duration in two patients with hypernephroma. Todd et al (5) treated 20 patients with measurable renal cancer using doses of 500 mg/m^2, with a dose escalation of 50 mg/m^2 every week. Of 18 evaluable patients, three (17%) achieved PR and one (6%) achieved CR. All responses occurred within 4 weeks and persisted for 1–5+ months. Seven (39%) patients had disease stabilization. Toxicity was more severe than was previously reported: nausea and vomiting occurred in 70%, mucositis occurred in 45%, myalgias occurred in 45%, weight loss occurred in 40%, neuralgias occurred in 35%, and diarrhea occurred in 15%. In a pharmacokinetic study of M-GAG by Liesmann et al (6), six patients with hypernephroma are mentioned but responses are not described.

The results of M-GAG in the present study were disappointing, particularly since 19 previously untreated patients had disease progression. The severity of side effects was unexpected, with the dose-limiting toxic effects being general malaise, fatigue, weakness, and myopathy. Recent pharmacokinetic studies by Hart et al (7) indicate a prolonged γ-half-life of M-GAG in patients with renal dysfunction which might explain the severity of the toxicity in the present study.

The response rate (CR + PR) with M-GAG in the present study is < 15% (95% confidence limits) in untreated patients and < 10% overall. The cumulative rate of response to M-GAG from reported cases and the present series is 11% (eight responses among 72 adequately treated patients with hypernephroma).

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Mitomycin C-Induced Skin Ulceration Remote From Infusion Site

Perivascular skin necrosis at the site of drug extravasation is a well-documented toxic side effect of mitomycin C administration. We report here two cases of skin ulceration in cutaneous areas remote from the drug infusion site. In both cases blood was drawn from a superficial vein, near the wrist, 1–3 hours before the infusion of mitomycin C. Mitomycin C at a dose of 5 mg/m², diluted to 100 ml, was then infused into an ipsilateral mid-forearm vein, by Soluset, through the side arm of a freely running saline infusion. The patients were observed throughout drug administration, checking for blood return, pain, or swelling. The infusion rate remained constant and no sign of infiltration appeared. Redness and itching occurred within 1 week at the initial blood drawing site which was several centimeters distal to the drug infusion site. This progressed to deep, painful tissue necrosis over the next several weeks. One patient was treated with Domeboro's solution soaks several times a day and healing gradually occurred over the next several months. The second patient died from his disease before healing occurred.

We suggest that mitomycin C, or other potentially sclerosing chemotherapeutic drugs, should not be administered into a limb that has had a recent venipuncture.

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Changes in Hair Pigmentation Associated With Cancer Chemotherapy[1]

We noted with interest the letter by Gauci and Serrou (1). To our knowledge, Korst and co-workers first described changes in hair when following cancer chemotherapy (2). Since our original description of cyclophosphamide alopecia in 1960 (3), in which changes occurring in the hair follicle were documented, we have found that pigmentary changes after regrowth are not uncommon occurrences. In 1962 (4), we described a woman whose light red hair was replaced by black hair on recovery from cyclophosphamide-induced alopecia. We have subsequently seen numerous patients in whom hair color changed from blond to black and vice versa. These changes occur more frequently in children and young adults. We have also reported (4) that grey hair is not infrequently replaced by hair of its former youthful color following cytostatic alopecia. After chemotherapy, change in hair color in a grey-haired patient can be due to regrowth of hair of a different color or to loss of the grey hair only, leaving the pigmented hair intact (4). Selective loss of more of the pigmented hair is not infrequently seen. In black patients the alopecia is often mild, but a change from the normal glossy black to a dull lighter-colored hair is common. In 1964 (5), we expanded our description of changes in the skin following cancer chemotherapy and propose that although the publications to which we refer are from 16 to 20 years old, they are still pertinent.

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Hexamethylmelamine in Mustard-Resistant Ovarian Adenocarcinoma

Hexamethylmelamine[1] has been shown in several trials to be active as a single agent in the treatment of ovarian cancer, especially in patients with no prior chemotherapy (1). However, variable results have been obtained with this drug when used in previously treated patients. Thus, we are reporting the experience of the Southeastern Cancer Study Group in mustard-resistant ovarian adenocarcinoma.

MATERIALS AND METHODS

Women were eligible if they had biopsy-proven stage III or IV ovarian adenocarcinoma, had failed previous chemotherapy with mustards (cyclophosphamide, chlorambucil, melphalan, and thiotepa), had at least one measurable lesion, and had a Karnofsky performance status of at least 40%. Eligible patients should have recovered from the hematologic toxicity of prior treatment, should not have significant impairment of kidney or liver function, and should have an estimated survival of at least 6 weeks.

Hexamethylmelamine (280 mg/m^2) was given orally in four approximately equal doses after meals and at bedtime on a long-term daily basis. Six weeks of treatment was considered an adequate trial. The dose was calculated to the nearest 100-mg capsule for each 3-week period.

If the granulocyte count decreased to 1500–2000/mm^3 or the platelet count decreased to 75,000–100,000/mm^3, a half dose of medicine was given. If the granulocyte count or platelet count was less than those values, treatment was stopped and then resumed at half dose when the counts were above those levels. Blood counts were done weekly and response was assessed every 3 weeks. Toxicity was determined as previously described (2). A partial response (PR) required at least a 50% reduction in the sum of the products of perpendicular diameters of measurable lesions.

RESULTS

Seventy-two patients were entered in the study; data were inadequate for six, 12 were ineligible (wrong diagnosis, four patients; uncertain primary, three; not recovered from prior treatment, one; no measurable lesion, two; and inadequate performance status, two), and five were inevaluable (lost to followup, two; refused treatment, two; and major protocol violation, one). Of 49 evaluable patients, one had a complete response (CR), seven had PRs, 15 had no change, 19 had disease progression, and seven died. The age range was 23–75 years (median, 57). Thus, the CR plus PR rate was 16% (eight of 49 patients). There were no responses in patients treated < 6 weeks, compared with one CR and seven PRs (21%) among 39 patients treated ≥ 6 weeks. Responses lasted 4, 5, 8, 16, 19, 19, 37, and 53 weeks. As expected, the major side effect was gastrointestinal toxicity, with 15 (31%) patients showing 1+ toxicity, 14 (29%) showing 2+, and four (8%) showing 3+. Neurologic toxicity was seen in four patients. Granulocyte toxicity was 1+ in four patients and 2+ in two patients. Platelet toxicity was more frequent, with 11 patients (22%) showing 1+ toxicity, three (6%) showing 2+, and one showing 3+. A dose reduction because of toxicity was required in 22 (45%) of the 49 patients.

DISCUSSION

The present study confirms previous reports that hexamethylmelamine is an active drug in ovarian adenocarcinoma (1,3–5) but agrees with the findings of Bolis et al (5) that the level of activity as secondary treatment in mustard-resistant cases is modest. In view of the frequency of troublesome gastrointestinal side effects and the low response rate, we think the long-term daily administration of hexamethylmelamine by itself to previously treated patients has only limited value.

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Invasive Thymoma Treated With Cisplatin

The treatment of invasive thymoma with chemotherapy has been reported in brief reports which were summarized by Boston (1). The value of cisplatin in metastatic thymoma was noted in a case report by Talley et al (2), where a good objective response was obtained. There are no other published reports of the use of cisplatin in this disease. We report here on a patient in whom cisplatin was used at a higher dose (ie, 100 mg/m^2) than was used by Talley et al (2). We obtained an excellent objective response suggesting that cisplatin has good activity in thymomas, that these tumors should be considered as responsive to chemotherapy, and that drug treatment should be added as the third modality in addition to surgery and radiation therapy. A cooperative multicenter trial on the use of chemotherapy in metastatic thymomas is indicated.

CASE REPORT

A 17-year-old male who had a routine chest roentgenogram following injury to his right shoulder was found to have a mediastinal tumor. He was completely asymptomatic. On June 24, 1975, he had a right exploratory thoracotomy, and a large multinodular tumor occupying the entire anterior superior mediastinum was found. Biopsy revealed an epithelial tumor, most probably a thymoma. Metastatic workup was negative. On December 15, 1975, alpha-fetoprotein was negative in the serum. From June 3, 1975, to August 7, 1975, he received 5000 rads to the mediastinum. A followup roentgenogram on May 29, 1978, showed no change in the size of the tumor. However, he developed a cold nodule in the lower portion of the left lobe of the thyroid which was excised on June 6, 1978, and proven to be metastatic thymoma. In December 1978 the mediastinal tumor had increased in size and his weight loss was 20 lbs. His tumor markers, alpha-fetoprotein and beta-subunit of human chorionic gonadotropin, were within normal limits on December 14, 1978. He was further treated with radiation therapy to the mediastinum from December 18, 1978, to February 3, 1979, when he received 2287 rads. On March 12, 1979, he was found to have a right neck tumor (5 × 5 cm in diameter) and a left axillary node and started receiving cisplatin by continuous infusion and mannitol diuresis. His renal function was normal. On April 4, 1979, the same dose of cisplatin (160 mg) was repeated. On May 1, 1979, he received 190 mg of cisplatin. On May 20, 1979, lung tomograms showed a decrease in the size of the tumor. In the neck tumor and left axillary node, there was an almost complete response (75%) to cisplatin. He refused further therapy and hence had recurrence of tumor. Chemotherapy was restarted on July 20, 1979, with doxorubicin and cisplatin. He received doxorubicin at a total dose of 670 mg until December 1979, which resulted in a partial response. In January 1980, he developed metastasis to bone at multiple sites and subsequently died on April 5, 1980.

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Tamoxifen in the Treatment of Malignant Melanoma

Since the report by Fischer et al (1) demonstrating the presence of estrogen receptors in human malignant melanoma, two reports have examined the influence of hormonal therapy in patients with malignant melanoma (2,3). Recently, Nesbit et al (3) have reported on the use of tamoxifen in 26 patients with advanced malignant melanoma. We have also used tamoxifen in five patients and wish to report evidence confirming the activity of tamoxifen in patients with stage III malignant melanoma refractory to "standard" chemotherapy.

Since July 1, 1979, we have treated five consec-

utive patients (four males and one female) with stage III melanoma (24–32 years old; median, 27) with tamoxifen at a dose of 10 mg twice a day. All patients had failed previous chemotherapy with DTIC alone or in combination and immunotherapy with BCG.

Estrogen- and progesterone-receptor activity was determined for all five patients (kindly performed by Dr. H. Rochman, University of Chicago, IL) to ascertain whether the presence or absence of receptor activity was at all predictive of response. A value of > 350 fmols/g of tissue was considered significant estrogen-receptor activity for the premenopausal female patients. Significant estrogen-receptor activity in males is unknown.

Two of the five tamoxifen-treated patients described below had partial responses (PRs). A 30-year-old white man had a malignant melanoma (level 4) excised from the left infrascapular area. An ipsilateral axillary node dissection was also performed and nodal metastases were found. Fifteen months later metastases to cervical, supraclavicular, and inguinal nodes, abdominal wall, and lung were discovered. There were no therapeutic effects from chemotherapy with vincristine, DTIC, BCNU, and hydroxyurea, which was followed by doxorubicin, cyclophosphamide, and procarbazine (also with no therapeutic effects). A biopsy of an involved cervical node with malignant melanoma revealed the presence of estrogen- and progesterone-receptor activity (297 and 164 fmols/g of tissue, respectively). Tamoxifen was initiated and resulted in complete regression of the abdominal nodules and a > 50% reduction of the lymphadenopathy and pulmonary metastases. The duration of the PR was 6 months.

A 28-year-old white man was found to have a nonpigmented lesion in the midepigastrum, a palpable left inguinal lymph node, and pulmonary nodules. Excision of the skin lesion and inguinal node revealed an amelanotic melanoma. Estrogen and progesterone receptors were present in significant quantities in the nodal metastases, 797 and 342 fmols/g of tissue, respectively. DTIC alone and BCG were without effect. Cyclophosphamide, vincristine, procarbazine, and prednisone were similarly ineffective. Tamoxifen therapy was started and resulted in a > 75% regression of the pulmonary metastases. This patient has continued to respond to tamoxifen for > 8 months.

The remaining three patients, despite the presence of estrogen and progesterone receptors in two of the three, failed to show any response to tamoxifen. The level of estrogen-receptor activity was 454, 891, and 43 fmols/g of tissue and the level of progesterone-receptor activity was 107, 233, and 7 fmols/g of tissue.

Our results confirm the activity of tamoxifen in stage III malignant melanoma as reported by Nesbit et al (3). It is clear that definite conclusions about the efficacy of tamoxifen and the predictability of estrogen-receptor activity in determining response cannot be made from the small number of patients we have treated to date. Similarly, Fischer could not correlate the presence of estrogen-receptor activity (four of 18 patients), with an objective response to diethylstilbestrol at a dose of 15 mg/day. The mechanism of tamoxifen-induced regression in malignant melanoma is unknown. Possible mechanisms include suppression of macromolecular protein and DNA synthesis by direct binding of tamoxifen with the estrogen receptor, altered pituitary peptide hormone production, or altered in vivo estrogen metabolism.

The treatment of stage III malignant melanoma is still experimental; thus, we urge investigators to begin comparative phase III trials with tamoxifen or diethylstilbestrol in patients with malignant melanoma. Pretherapy and posttherapy endocrinology evaluation in such patients may help to unravel the mechanism of hormonal-induced regression of malignant melanoma.

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Update on In Vitro Testing of New Drugs in Ovarian Cancer and Melanoma

In the January/February 1981 issue of *Cancer Treatment Reports* (Vol. 65, No. 1–2, pp 1–12), our

group published a report of its initial years' experience using the in vitro clonogenic or human tumor stem cell assay for in vitro phase II screening in ovarian cancer and melanoma. Between the time of original submission of the paper (February 1980) and the present (April 1981), substantial additional data have accumulated on in vitro sensitivity for a number of the agents which we had tested. Table 1 below was developed using the criteria delineated in the paper and incorporates and adds to the data summarized in the paper. These in vitro studies now encompass tests on tumor specimens from 98 patients with ovarian cancer and 46 patients with melanoma. The additional data suggest that vinca alkaloids (particularly vinblastine) have significant activity in both neoplasms. Additionally, dihydroxyanthracenedione may have use in ovarian cancer and AMSA may have use in melanoma. Such leads for the latter two agents may be particularly useful for initiating clinical trials in patients who have not had prior exposure to anthracyclines. In addition to these agents, we have also recently been testing the new amino anthraquinone derivative, anthracenedicarboxaldehyde. We have found evidence of in vitro activity in one of eight patients (13%) with ovarian cancer and six of 13 patients (46%) with melanoma. We have also, in preliminary studies, seen close clinical correlations between in vitro and in vivo sensitivity with anthracenedicarboxaldehyde. These additional data may prove useful to the readers in further assessing the role of in vitro assay as an alternative to the standard clinical phase II testing approach for identifying antitumor activity of new agents.

TABLE 1.—Sensitivity of tumor colony-forming units to 1-hr exposure to single drugs*

Drug	No. of sensitive cultures/No. tested (%)	
	Ovarian cancer	Melanoma
Mitomycin C	1/14 (7)	0/1 (0)
Pentamethylmelamine	2/15 (13)	NT
Vindesine	1/15 (7)	8/28 (29)
Vinblastine	19/85 (22)	5/16 (31)
Methyl-GAG	2/13 (15)	1/6 (17)
PALA	0/8 (0)	0/7 (0)
AMSA	4/36 (11)	8/30 (27)
Dihydroxyanthracenedione	4/21 (19)	1/3 (33)
Anthracenedicarboxaldehyde	1/8 (13)	6/13 (46)
Thymidine	NT	0/7 (0)

*For the purposes of this comparison, our operational definition of sensitivity is defined as a > 70% reduction in survival at 10% of the pharmacologically achievable C × t. NT = not tested.

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Phase II Trial: Mitomycin C and Methotrexate Combination Therapy for Advanced Colorectal Carcinoma

Treatment of metastatic colorectal carcinoma remains unsatisfactory, with combination chemotherapy showing no major survival advantage over single-agent therapy (1,2). Recent interest in possible in vitro synergism (3) between mitomycin C, an active agent in colorectal carcinoma (4), and methotrexate prompted a phase II trial in previously treated, disseminated, histologically documented colorectal carcinoma.

Patients with a Cancer and Leukemia Group B performance status of ≤ 3 (ambulatory), measurable disease as defined previously (1), a total wbc count $\geq 4000/\mu l$, a platelet count $\geq 150{,}000/\mu l$, and a serum creatinine < 1.2 mg/dl were offered the combination chemotherapy. Study patients were initially evaluated by serum liver enzymes, creatinine, physical examination, chest roentogram, and a liver scan or computerized axial tomographic (CAT) scan in the seven cases of pelvic-mesenteric disease. All patients had a biweekly examination, weekly cbc and platelet count, and monthly serum creatinine measurement. If serum creatinine increased to > 1.4 mg/100 ml, the patient was removed from the study. Areas of measurable disease defined on liver or CAT scan were repeated at 8-week intervals.

Patients were evaluated for response, by defined criteria (2). Mitomycin C (10 mg/m^2) was given by iv bolus every 5 weeks, and methotrexate (20 mg/m^2) was given by iv bolus weekly. If the wbc count was 3000–3500/μl or the platelet count was 75,000–100,000/μl on the day of treatment, the dose was reduced to 50% of the initial dose; if hematologic depression was greater, the treatment was delayed for 1 week.

Twenty-eight consecutive patients were entered in the study (table 1). Two patients were inevaluable. Of the evaluable patients (15 males and 11 females), the average age was 58 years (range, 32–79) and the average performance status was 2. The total average doses ($\pm$ SD) were 86% $\pm$ 17% of the theoretic maximum dose for mitomycin C and 75% $\pm$ 22% of the theoretic maximum for methotrexate.

Significant hematologic toxicity occurred in 69% of the patients, with two life-threatening septic episodes; severe stomatitis occurred in 30% (table 1).

This trial did not demonstrate in vivo synergism between mitomycin C and methotrexate in previously treated patients. The major response rate was only 15% (95% confidence limits, 1%–29%). The mean duration of response was 16 weeks and occurred only in three patients with liver metastasis and one patient with mesenteric involvement.

TABLE 1.—Patient characteristics and treatment results

	No. of patients (total: 26)
Dominant disease	
Liver	16
Lung	3
Pelvic/mesenteric	7
Prior therapy	24
5-FU	24
Nitrosourea	2
Radiation	3
Average total cycles of protocol given/patient (range): 2.8 (1–7)	
Response	
Complete	0
Partial	4 (15%)*
Toxicity	
Hematologic†	18
Stomatitis $\geq 2+$	8
Septic episodes	2

*Duration of responses (wks): 4, 11, 38, and 13.

†Nadir wbc and platelet counts (mean $\pm$ SD): 5300 $\pm$ 2500 and 113,000 $\pm$ 60,300, respectively.

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Phase II Study of Maytansine in Advanced Breast Cancer[1]

After a phase I clinical trial had suggested possible activity of maytansine against human breast cancer, we began a phase II study in patients with advanced disease (1).

Sixteen extensively pretreated patients with histologically confirmed metastatic breast cancer were treated by rapid iv infusion with maytansine (0.6 mg/m²) daily for 3 consecutive days every 3 weeks. Requirements for admission to the study included the presence of progressive evaluable disease; expected survival of at least 2 months; at least semiambulatory performance status; and adequate renal, hepatic, and bone marrow functions.

Following the initiation of treatment, maytansine doses were modified for any excessive toxicity: hematologic, mucosal, neurologic, hepatic, or gastrointestinal.

None of the 15 patients with followup adequate for evaluation experienced objective tumor regression; however, two patients experienced minor degrees of tumor shrinkage, each for 6 weeks. Stability of disease for at least 6 weeks was observed in ten patients, but only in two of these did the stability persist longer (for 9 weeks in one patient). Three patients experienced immediate disease progression following the initial course. Median survival (Kaplan-Meier) for the 16 patients who received maytansine was 166 days. Among the 15 patients for whom any toxic effects were adequately described, only one completed her maytansine trial without any drug toxicity (table 1).

As previously observed by others, the most common toxic effects of maytansine are gastrointestinal, with diarrhea the most troublesome. Of particular interest, however, was our observation of constipation in some of these same patients. Cabanillas et al (2) have also reported constipation in some of their patients receiving maytansine on a 3-day schedule similar to ours, and a few of their patients developed full-blown ileus. Perhaps maytansine produces vinca alkaloid-like intestinal neurotoxicity in addition to the more commonly observed intestinal irritation and diarrhea.

Because of the expected poor therapeutic response of late-stage, multiply treated patients such as those in this trial, the relative lack of therapeutic efficacy observed should not eliminate maytansine from further study in less-advanced breast cancer.

[1]Supported in part by contract N01-CM-57044 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

TABLE 1.—Patient characteristics and treatment results

Age	Performance status*	No. of previous drug treatment regimens	Response to maytansine	No. of courses of maytansine treatment	Toxic effects
58	1	5	Stable	3	Diarrhea, myalgia
80	2	5	Improved	3	Nausea & vomiting, constipation, diarrhea, myalgia
53	3	4	Stable	2	Leukopenia (3300 cells/μl), stomatitis, nausea & vomiting, constipation, diarrhea, paresthesias
68	2	4	Stable	2	Stomatitis, nausea & vomiting, myalgia, dermatitis, phlebitis
67	0	4	Stable	2	Psychosis (treatment stopped due to toxicity)
58	1	5	Stable	2	Stomatitis, constipation, diarrhea
63	1	5	Progression	1	None
42	2	5	Stable	3	Nausea & vomiting, myalgia
50	2	5	Stable	2	Leukopenia (3900 cells/μl), nausea, diarrhea, paresthesias
57	1	5	Stable	2	Nausea, constipation, diarrhea
58	1	3	Improved	3	Fever, diarrhea, myalgia, paresthesias
73	1	7	Progression	1	Nausea & vomiting
72	1	6	Stable	2	Nausea, constipation, diarrhea
56	1	4	Stable	2	Paresthesias, thrombocytopenia (100,000 cells/μl)
59	0	4	Progression	1	Bladder spasm, nausea, diarrhea, leukopenia (3700 cells/μl)
47	2	3	Not evaluable	2	Not evaluable

*Eastern Cooperative Oncology Group criteria.

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April 2, 1981

Phase II Study of Chlorozotocin in Advanced Breast Cancer[1]

Chlorozotocin is the 2-chloroethyl analog of the glucose-substituted *N*-methyl-*N*-nitrosourea anticancer antibiotic, streptozocin (1). A previous phase I clinical study identified toxic but tolerable effects of chlorozotocin at doses of 150 mg/m² every 6 weeks (2).

Twenty-five extensively pretreated patients with histologically confirmed metastatic breast cancer were treated by rapid iv infusion with chlorozotocin (150 mg/m²) every 6 weeks. Requirements for admission to the study included the presence of progressive evaluable disease; expected survival of at least 2 months; at least semiambulatory performance status; and adequate renal, hepatic, and bone marrow functions.

[1]Supported in part by contract N01-CM-57044 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

TABLE 1.—Patient characteristics and treatment results

Age	Performance status*	No. of previous drug treatment regimens	Best response to chlorozotocin	No. of courses of chlorozotocin treatment	Toxic effects
80	2	4	Progression	1	Thrombocytopenia (93,000 cells/μl)
35	3	2	Progression	1	Not evaluable
50	2	3	Progression	1	Thrombocytopenia (31,000 cells/μl)
52	3	4	Progression	1	Nausea & vomiting
50	1	3	Progression	1	None
54	1	6	Progression	1	Not evaluable
54	1	4	Progression	1	Thrombocytopenia (60,000 cells/μl)
53	2	4	Progression	1	Not evaluable
63	1	4	Progression	1	Nausea & vomiting, leukopenia (3100 cells/μl) & thrombocytopenia (79,000 cells/μl)
63	2	5	Progression	1	None
64	1	3	Progression	1	Not evaluable
58	1	4	Progression	1	Leukopenia (3500 cells/μl)
38	1	3	Progression	1	None
35	2	3	Progression	1	Not evaluable
68	2	5	Stability	2	None
56	1	5	Progression	1	None
59	1	5	Progression	1	Leukopenia (3600 cells/μl)
58	2	6	Progression	1	Nausea & vomiting
56	2	5	Progression	1	Not evaluable
68	3	5	Progression	1	Thrombocytopenia (128,000 cells/μl)
67	0	5	Progression	1	Leukopenia (2100 cells/μl)
58	1	6	Progression	1	None
50	2	6	Progression	1	None
53	3	5	Stability	2	Leukopenia (2800 cells/μl) & thrombocytopenia (43,000 cells/μl)
58	2	4	Stability	2	Leukopenia (3000 cells/μl), nausea & vomiting

*Eastern Cooperative Oncology Group Criteria.

Following the initiation of treatment, chlorozotocin doses were modified for any excessive hematologic or gastrointestinal toxicity.

None of the 25 patients experienced objective tumor regression, and none showed improvement. Only three patients had stability of disease at the time of initial re-evaluation, and only these three received a second dose of chlorozotocin. Twenty-two patients experienced immediate progression of disease or symptomatic deterioration due to disease, and five of them died of metastatic cancer within 6 weeks following their initial doses of chlorozotocin. Median survival (Kaplan-Meier) for the 25 patients who received chlorozotocin was 108 days. Among the 19 patients in whom hematologic toxicity could be satisfactorily assessed, seven experienced no significant toxicity (table 1).

Our regimen involving rapid iv infusion of 150 mg/m^2 of chlorozotocin was, however, within the range suggested from one phase I study (2) and higher than that recommended by the authors of another (3). Furthermore, some of our patients did develop clinically significant myelosuppression. Although the patients studied were representative only of a later stage, previously treated breast cancer patient population, the lack of clinical benefit observed during this trial dissuades us from further study of chlorozotocin in advanced breast cancer.

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Phase II Study of High-Dose Methotrexate in Patients With Unresectable Metastatic Osteosarcoma[1]

Following a report of regression of pulmonary metastases from osteosarcoma in 1972 by Jaffe (1), the use of high-dose methotrexate (HDMTX) with leucovorin rescue has become an accepted treatment for metastatic osteosarcoma (2). As discussed by Pratt et al (3), however, published data concerning methotrexate for advanced osteosarcoma are quite limited; thus we present here an additional series of sixteen cases treated with this drug.

During 1977–1980, patients with unresectable metastatic osteosarcoma received HDMTX according to this protocol which required that their renal, hepatic, and bone marrow functions were normal and that they had performance status estimates above level 4 (Eastern Cooperative Oncology Group). Thirty minutes after the iv injection of vincristine (2 mg/m^2; maximum single dose, 2 mg), a 6-hour iv infusion of HDMTX (3 g/m^2) was begun. Two hours after completion of this infusion, calcium leucovorin (15 mg every 3 hours) was administered via an alkalinized continuous iv infusion for 8 doses. Following the initial day of leucovorin rescue, this same dose was added at 6-hour intervals until the blood methotrexate concentration reached 1×10^{-7} M. The dose of HDMTX was increased, if tolerable, to 6 g/m^2 and then to 7.5 g/m^2 at the times of re-treatment at 3-week intervals, and treatment was continued until definite disease progression occurred. Doses were also escalated in four patients despite overt disease progression and in no case was this escalation effective. Thirteen of these patients had not previously received any cytotoxic drug treatment (table 1).

One of these 16 patients achieved objective tumor regression for 4 months, and seven others experienced disease stability for 3 weeks or more. Following failure of HDMTX, four of these patients received protocol treatments combining cyclophosphamide, doxorubicin, and cisplatin; two patients achieved partial tumor regression. Of the two patients who failed HDMTX as secondary treatment, one had achieved complete tumor regression previously on the cyclophosphamide-doxorubicin-cisplatin treatment. We have not been favorably impressed with the efficacy of HDMTX in unresectable metastatic osteosarcoma.

[1]Supported in part by contract N01-CM-57044 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

TABLE 1.—Patient characteristics and treatment results

Age	Sex	Histologic type and site of origin of tumor	Previous chemotherapy*	Highest MTX dose (g/m²)	Best response†
43	M	Grade 4 osteosarcoma of scapula (associated with Paget's disease)	None	6	S
16	M	Grade 4 osteoblastic osteosarcoma of femur	None	6	S
19	M	Grade 4 osteoblastic osteosarcoma of tibia	None	6	P
16	M	Grade 4 telangiectatic osteosarcoma of femur	None	6	P
9	M	Grade 4 telangiectatic osteosarcoma of femur	None	7.5	P
51	F	Grade 4 osteosarcoma of vertebra	None	3	S
26	M	Grade 4 fibroblastic osteosarcoma of pelvis	None	7.5	PR for 4 mos
17	M	Grade 4 telangiectatic osteosarcoma of tibia	MeCCNU, CTX-DOX-CDDP	3	P
65	M	Grade 3 osteosarcoma of femur	None	6	P
32	F	Grade 2 osteoblastic osteosarcoma of ethmoid	None	6	S
23	M	Grade 4 small cell osteosarcoma of femur	None	3	P
56	M	Grade 4 osteosarcoma of femur	None	3	S
17	M	Grade 4 chondroblastic osteosarcoma of pelvis	None	6	S
70	M	Grade 4 osteosarcoma of femur	MTX as adjuvant treatment	7.5	S
16	M	Grade 4 osteoblastic osteosarcoma of femur	None	3	P
16	M	Grade 4 osteoblastic osteosarcoma of femur	Pyrazofurin, CTX-DOX-CDDP	3	P

*MeCCNU = methyl-CCNU; CTX = cyclophosphamide; DOX = doxorubicin; and CDDP = cisplatin.
†S = stable disease; P = progressive disease; PR = partial remission.

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A Preliminary Report of Mitotane Therapy of Advanced Renal and Prostate Cancer

Mitotane (*o,p'*-DDD) is an active antineoplastic agent for advanced adrenal cortical carcinoma (1) and for some nonadrenal malignancies (2). It inhibits adrenal steroidogenesis (3) and, at 1–10 μg/ml, levels attainable in human plasma (4), inhibits the in vitro growth of human renal carcinoma cells, astrocytoma cells, and fibroblasts (T. Hogan, unpublished data).

In 1978, we initiated a phase II study using low doses of mitotane in patients with advanced renal or prostate cancer. Concurrent glucocorticoid therapy was used in all patients (prednisone [10 mg/day] or cortisone [25 and 12 mg/day]); mineralocorticoid therapy was used in 16 (fludrocortisone [0.1–0.5 mg/day]).

To date, 20 patients have been studied (table 1). All 12 patients with renal carcinoma had measurable metastatic disease; seven had failed prior chemotherapy and three had failed prior hormone therapy. All eight patients with prostate carcinoma had evaluable osteoblastic bone metastases; eight had failed diethylstilbestrol treatment, eight had failed estramustine phosphate treatment, and three had had orchiectomy. No objective response has been seen in the 12 patients with renal cancer. However, one patient with prostate cancer who had failed orchiectomy, diethylstilbestrol treatment, and estramustine phosphate treatment has had decreased bone pain, stable serum acid phosphatase, and stable bone scans lasting 4 months.

During the first month of low-dose mitotane therapy, acute toxic reactions (nausea, vomiting, or dermatitis) did not occur; after 1 month, toxic effects developed in most patients: gastrointestinal (two), CNS (six), and dermatitis (two). With exogenous steroid replacement, clinical adrenal insufficiency did not occur.

TABLE 1.—Clinical profile of 20 patients treated with mitotane

Age (yrs)	Sex	Performance status*	Days treated/survival time†	Dose (g/day)		Toxicity‡	Results
				Initial	Maximum		
			Renal cancer				
46	F	3	17/45	3	3	None	Disease progression
22	M	1	20/20	3	3	None	Disease progression
53	M	3	20/20	3	3	None	Disease progression
50	M	1	33/95+	3	4	GI/CNS	Disease progression
68	M	1	42/263	3	3	GI	Disease progression
48	M	1	46/61	3	3	None	Disease progression
62	M	1	50/86	3	3	None	Disease progression
68	M	2	55/166	3	3	GI/CNS	Stable (2 mos)
67	M	2	59/73	3	3	GI	Disease progression
65	M	0	65/156	3	3	GI/CNS/cutaneous	Stable (2 mos)
57	F	2	87/255+	3	3	GI/CNS	Stable (2 mos)
48	M	1	392/593+	3	5	CNS	Stable (5 mos)
			Prostate cancer				
77	M	1	20/159	3	3	Unknown	Disease progression
63	M	3	22/403	3	3	Unknown	Disease progression
57	M	3	24/24	3	3	Unknown	Disease progression
53	M	3	28/28	3	3	Unknown	Disease progression
55	M	1	66/189	3	4	None	Disease progression
63	M	1	82/135	3	5	CNS/cutaneous	Disease progression
76	M	2	90/238	3	5	None	Disease progression
71	M	2	201/365+	3	3	CNS	Improved (4 mos)

*Eastern Cooperative Oncology Group criteria.

†Days from initiation of mitotane treatment.

‡When not attributable to other clinical factors, *CNS* toxicity was the gradual onset of fatigue, somnolence, or depression; *GI* toxicity was anorexia, nausea, vomiting, or diarrhea; and *cutaneous* toxicity was the sudden onset of any dermatitis. All drug-related toxic effects abated after therapy was stopped.

Mitotane does not suppress bone marrow, liver, or renal function, is lipid-soluble with good CNS entry (5), and is well-tolerated if exogenous steroid replacement is used. It may be useful adjunctive therapy and deserves further clinical trial in patients with nonadrenal malignancies.

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ANNOUNCEMENTS

Advanced Annual Seminar in Urologic Surgery: Prostate and Bladder Cancer

San Francisco, California, October 9–10, 1981

The University of California School of Medicine will present a program entitled "Advanced Annual Seminar in Urologic Surgery: Prostate and Bladder Cancer" on October 9–10, 1981, at the Golden Gateway Holiday Inn, San Francisco. This seminar is designed to provide the most recent in-depth basic scientific and clinical information concerning bladder and prostate carcinoma. Controversial areas of clinical management will be a major thrust.

Prostate carcinoma topics include pelvic lymph node dissection and its place in diagnosis and treatment, the role of radiation therapy in curative treatment, current diagnostic and staging techniques, indications and techniques for radical prostatectomy, and hormonal and cytotoxic therapy.

Bladder carcinoma topics include problems of carcinoma in situ, the role of preoperative radiation therapy, significance of pelvic lymphadenectomy and urethrectomy, current advances in diagnostic techniques, and chemotherapy.

Co-chairmen are Jack W. McAninch, MD, and Richard D. Williams, MD. The program meets the criteria for AMA/CMA Category I credit.

For further information, please write or call: Extended Programs in Medical Education, University of California School of Medicine, Rm 569-U, Third and Parnassus, San Francisco, CA 94143 (415–666-4251).

Medical Oncology Review

Honolulu, Hawaii, October 12–16, 1981

The University of Hawaii Cancer Center and The Royal Australasian College of Physicians are sponsoring a medical oncology review (American College of Physicians course No. 019) to be held October 12–16, 1981, at the Princess Kaiulani Sheraton Hotel, Honolulu, Hawaii. The course will be directed by Thomas C. Hall, MD, with Thomas Hurley, MD, as co-director.

The review is designed for persons preparing for the medical oncology board examination, internists desiring a comprehensive refresher course, and oncologists desiring a rigorous update. Clinically relevant aspects of causation, growth, biology, diagnosis, treatment, and rehabilitation from the view of the internist will be reviewed. Methods of instruction will include lectures with a prepared outline of material and slides, luncheons with the professors, case presentations and discussions, panel discussions, posters, film strips, movies, and pre- and post-quiz.

The ACP certifies that this Continuing Medical Education course meets the criteria for Category I credit toward the AMA Physicians Recognition Award, provided it is used and completed as designed.

Strenuous efforts have been made to obtain inexpensive travel and accommodations. Reserved special rate airfares have been arranged on a regular carrier from the US mainland and Australia. For reservations contact Crimson Travel, 39 Boylston St, Cambridge, MA 02139.

For further information about the course contact: Dr. Thomas C. Hall, Community Cancer Program of Hawaii, 1236 Lauhala St, Honolulu, HI 96813 (telephone: 808–548-8422).

CORRECTION

Due to printer's error, figures 1–3 in the article "Radionuclide Angiography to Predict Patient Responses to Hepatic Artery Chemotherapy" by Kaplan et al (Vol. 64, No. 12, December 1980, pages 1217–1222) were not of sufficient quality to convey necessary information. They are reprinted below with their legends.

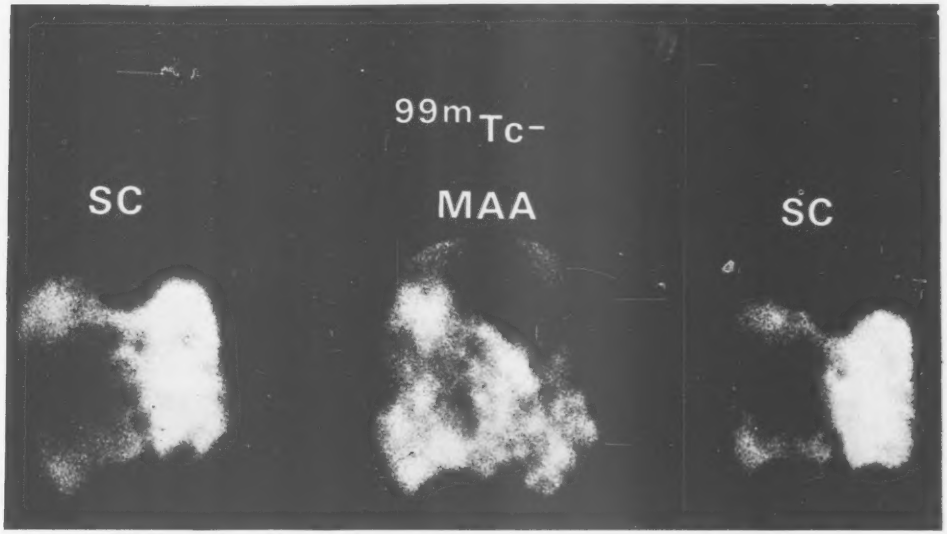

FIGURE 1.—^{99m}Tc-SC liver scan (left) shows large focal defect in right hepatic lobe. Perfusion study (center) indicates adequate flow to entire liver. Followup radiocolloid liver scan (right) shows marked regression in hepatic size (patient 2).

continued

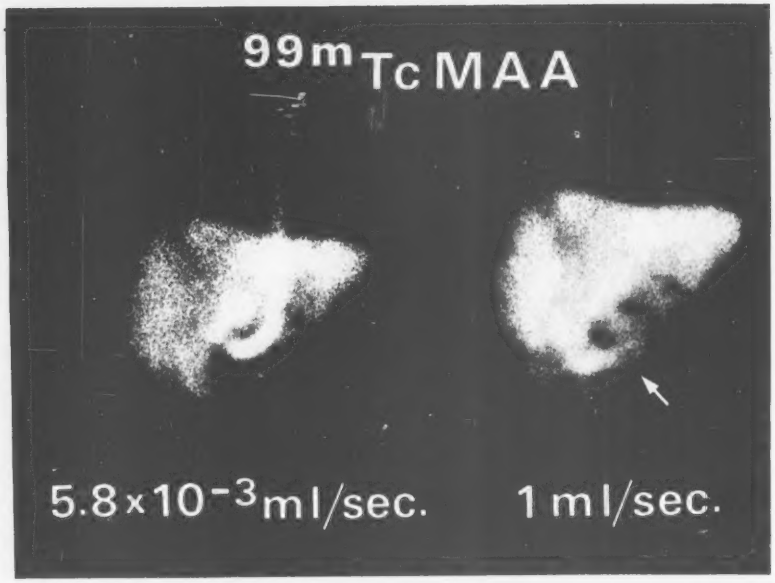

FIGURE 2.—At flow rate employed for infusion therapy (left) there is inadequate perfusion of focal defect at inferior margin of liver. Higher flow rate indicates good perfusion of this lesion (arrow) (patient 7).

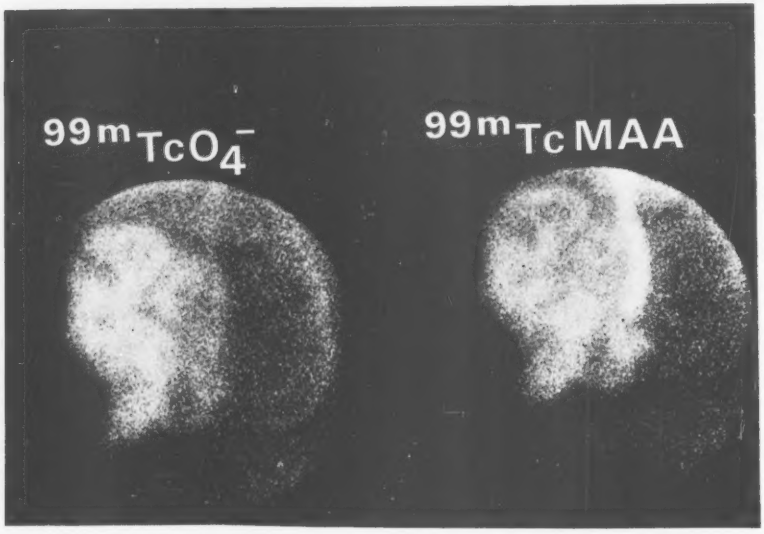

FIGURE 3.—Infusion of $^{99m}TcO_4^-$ and ^{99m}Tc-MAA at 5.3×10^{-3} ml/sec is seen to produce identical patterns of intrahepatic distribution (patient 10).

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